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STUDIES IN CELLULAR PATHOLOGY

I. EFFECTS OF CANE GALL BACTERIA UPON GALL TISSUE CELLS OF THE BLACK RASPBERRY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 464

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(WITH PLATE I AND ONE FIGURE)

Introduction

It is the purpose of this paper (1) to present procedures and criteria by which the bacteria which induce and are present in cane galls of black raspberry (*Rubus occidentalis* L.) may be differentiated from all bodies which occur naturally within the cells of this plant; (2) to describe the locus and migration of the parasite within the tissues of the galls which it incites; and (3) to describe some of the effects of the organism upon the cells of this plant. Throughout this research the major emphasis was placed upon critical comparison of the cytoplasms of healthy and pathic cells and upon the differentiation of bacteria from the bodies of like form and size which occur in the cytoplasm of this plant.

Cane gall is a bacterial disease of bramble fruits which occurs widely in the United States and which has been reported from Europe (**104, 105**). On the black raspberry in the Great Lakes area the bacterium incites numerous white, soft, parenchymatous galls upon the surfaces of the aerial portions of the fruiting canes during the fruiting season. It has been assumed by many pathologists that this disease is induced by the crown gall organism *Phytomonas tume-*

faciens (*Bacterium tumefaciens* Smith and Town) Bergey *et al.* Conclusive proof of the identity of the cane gall and crown gall diseases of bramble fruits is wanting. Available evidence by BANFIELD (**2, 3**) and PINCKARD (**82**) suggests that the organisms inducing these diseases are distinct.

Materials and methods

The material studied has been of three types: (a) galls of various ages found occurring naturally on black raspberry and collected from plantations in the Great Lakes area; (b) galls induced on black raspberry plants in the greenhouse by inoculation with pure cultures of the cane gall bacterium; and (c) root tip and apical stem meristems, for purposes of comparison, from plants free from this disease. Comparative study of fresh and fixed tissue was made in all phases of the research.

The tissues to be studied in the fresh condition were collected immediately before use from plants in the greenhouse. At all times during preparation or examination they were kept immersed in sterile sucrose solutions of 5–10 per cent concentration or in sterile water. Sucrose solutions of 7.5 per cent concentration induced no conspicuous changes in the uncut cells of fresh sections during observation periods limited to a few hours, whereas 5 or 10 per cent solutions induced appreciable changes. Cells mounted in sterile water quickly degenerated. Sections were prepared freehand from tissue fragments held in pith soaked in the appropriate mounting fluid.

Sections were mounted under thin covers on glass slides which were placed on the stage of the microscope in oil immersion contact with a Zeiss aplanatic condenser (n.a. 1.4). A Zeiss appochromatic water immersion objective (70× n.a. 1.25) with compensating oculars (10, 15, or 20) was used for studying the finer cellular detail. Light of sufficient intensity was obtained from a 4.5 ampere carbon arc, the rays of which were condensed by a 3-liter Florence flask which was placed between the arc and the microscope and which contained cupra ammonia solution of desired color intensity. A second filter of blue ground glass was placed immediately under the condenser of the microscope.

Various fixation and staining methods were used for more perma-

nent mounts. The standard methods used in the study of mitochondria were found to give the best fixation of the bacteria in the tissues of diseased plants. Among these the fluids of REGAUD IV b (**52, 51, 86**), NEMEC (**78, 68**), ERLICKI (**52, 51**), MOTTIER'S modification of BENDA (**71**), and the following BENDA-ERLICKI technique gave the best fixation of the bacteria.

BENDA-ERLICKI FIXATION

SOLUTION A.—Chromic acid, 1 per cent		17 cc.
	Osmic acid, 2 per cent	3 cc.
	Acetic acid, glacial	add 2 drops
SOLUTION B.—Copper sulphate, 2 per cent		1 volume
	Potassium dichromate, 5 per cent	1 volume

Mix just before using.

Fix 36 hours in solution A, wash two hours, fix 24 hours in solution B, wash 24 hours in running water, dehydrate and imbed.

These same fluids, particularly the REGAUD, faithfully preserved the structure of the cytoplasm as seen in fresh uncut cells mounted in isotonic sucrose solution, and were found adequate for the study of the effects of the bacteria upon the structure of the cytoplasm. Haidenhain's iron-alum haematoxylin gave intense and sharply selective staining of the bacteria and chondriome.[1]

This stain was used almost exclusively in this work. The selective staining of bacteria is discussed in a later section.

I. Differentiation of bacteria from cell contents

A. NON-INFECTED TISSUES

a. Microchemical tests

Microchemical tests were made to ascertain the nature of three classes of ergastic bodies usually encountered in abundance in certain cells of plants affected or unaffected by the cane gall bacterium, and in the cells of galls induced by the cane gall or crown gall organism. These three classes of bodies were: (a) yellow globular and granular tannin bodies precipitated by the fixing fluids employed, (b) starch grains, and (c) fat globules. Because of their similarity in

[1] A term introduced by GUILLERMOND (**32**) to designate mitochondria and plastids collectively.

size and form these bodies at times might be confused with bacteria, but generally they are characteristically different. Since these three classes of bodies appeared to account for all cellular inclusions usually encountered aside from mitochondria, vacuoles, plastids, or bacteria, microchemical tests other than those deemed necessary to establish the nature of these bodies were not made.

1. TANNIN.—Pale yellowish to brown granules and globules of various size are present in abundance in many of the cells of material fixed in the fluids used for preserving mitochondria by the REGAUD, BENDA, or NEMEC techniques respectively. These bodies may be dark brown or black as a result of the action of osmic acid after fixation in BENDA'S fluid. They may be stained black after immersion in haematoxylin and differentiation for mitochondria, when the tissue is fixed in any of the fluids used in this work for the preservation of the chondriome. In size they vary from granules 1–5 μ in diameter to single globular structures which may occupy practically all the space within the walls of particular cells. Granules may be imbedded within such globules. Unlike the other ergastic inclusions discussed, tannin appears to occur more abundantly in the vacuole of the black raspberry cell.

Tannin bodies occur chiefly in the ray parenchyma cells of the phloem and pericycle, and in the cortical cells of the black raspberry stem. Often most of the cells in certain areas are filled with these bodies. They are found in like abundance in many of the cells of certain areas of gall tissue in which cell division does not occur and in which the cells appear to have differentiated. Such bodies have not been observed in the cells of unstained fresh sections. They appear, however, as globules of various dimension within the same classes of cells in which they occur in fixed material in sections of fresh tissue that have been immersed in 8 per cent formaldehyde solution, or in iodine potassium iodide solution. They appear as globules and granules in sections of fresh tissue treated with 1 per cent chromic acid or potassium dichromate solution or in the fixing fluids of BENDA, NEMEC, REGAUD, or ERLICKI. These granules and globules stain light to dark blue black in 10 per cent ferrous sulphate, green to greenish black in 10 per cent ferric chloride, and turn brown or blacken when in contact with osmic acid vapor. These are reac-

tions characteristic of tannin (**101**) and these bodies are therefore regarded as tannin bodies precipitated by the fixing fluids employed.

When dilute solutions of neutral red are added to the mounting medium, numerous globules within the cytoplasm of certain cells appear deep red or violet. A granular precipitate of like color can be seen in many large vacuoles and a diffuse red color without precipitates occurs in others. Large globules which absorb the red color also are seen in other vacuoles. It appears that these globules and granules which stain in living cells immersed in dilute solutions of neutral red are similar if not identical to those globules and granules which are rendered visible in fresh cells by bathing the cells in iodine or formaldehyde solutions, and which stain with ferric salts, or blacken in osmic vapor. These color responses have been attributed to the presence within the vacuole of phenolic compounds of the tannin group by the DANGEARDS (**16, 19**), GUILLIERMOND (**32–42**), and DUFRENOY (**21–28**). KLERCKER (**50**), LLOYD (**59, 60**), MICHEL-DURAND (**30, 31**), MCNAIR (**67**), and GUILLIERMOND (**41**) report tannin as commonly occurring in the vacuoles of certain cells of various plants. Tannin bearing and non-tannin bearing vacuoles may exist side by side in the same cell (**50**).

2. STARCH.—The relatively large (2–5 μ) spheroidal or irregularly rounded bodies of high refractivity which are abundant at times in the living cells of sections of root tip or gall tissue impart a decidedly granular appearance to these cells, and have the following properties: (a) double refraction to polarized light, (b) digestibility in diastase, and (c) deep blue black staining with iodine potassium iodide solution. These bodies range in size from 0.4 to 5 μ. Frequently their refractivity is only slightly greater than that of mitochondria. Similar bodies but of low refractivity give no blue black color when treated with iodine solution although they may give a slight violet color with iodine solution after treatment in chloral hydrate. Because of these properties they are considered to be plastids which contain a varying quantity of starch as indicated by their refractivity and size.

The tissues of some galls are rich in starch grains, meristematic and degenerating areas excepted. In the early stages of development, galls usually contain very little starch. The ray, pith collen-

chyma, pericycle, cortex, and phloem parenchyma cells of first year canes are rich in starch grains. Only a few scattered cells of the pith contain starch grains and then in but small numbers.

3. FAT.—Small spherical bodies of high refractivity are characteristically present in the cytoplasm. If fresh sections of root tip or gall tissue are treated with Sudan III as described by TUNMANN-ROSENTHALER (**101**), numerous bright red globules ranging in size from 0.2 to 2.0 μ appear in the cells. If these refractive globules are observed under the microscope as a solution of Sudan III in 70 per cent alcohol is introduced under the coverslip, they can be observed gradually absorbing the dye. Sections treated in alkanin react similarly. The bodies in question have an olive cast when in focus, and their shadow when out of focus is markedly intensified after exposure to osmic acid fumes, which causes the globules to appear dark brown or black. When seen in the vacuole or in water they have diffraction rings or halos about them, but this halo does not appear when they are in the cytoplasmic ground substance. After treating fresh sections of root tip or gall tissue with ether or chloroform, neither these minute highly refractive bodies nor mitochondria can be seen, nor can they be stained in the cells of such tissue by osmic acid vapor, Sudan III, or alkanin. Because of these reactions these minute spherical bodies of higher refractivity than the mitochondria are assumed to be of fatty nature. They have been described by DANGEARD (**16, 17, 18**) and by GUILLIERMOND (**34, 36, 37, 39, 40, 42**) as being common constituents of the cytoplasm of most if not all plant cells. They have been variously termed microsomes, spherosomes, cytosomes, lipoid granules, fat globules, etc.

b. Cells of root tip

Since cane galls are largely composed of meristematic cells, the apical meristem of root tip or stem was used for comparative studies on healthy tissues. Root tips were used more extensively because they are readily and abundantly obtained by layering the tips of first year canes during midsummer to late autumn, and because they, like gall cells generally, are devoid of chloroplasts.

Living cells of root tips as observed in fresh sections mounted in 7.5 per cent sucrose solution are characterized by the following gen-

eral features. The cell walls appear as luminous sharp linear bands of appreciable thickness, usually as parallel pairs with a faint dark line, the middle lamella, between. The cytoplasm of the cell appears as a translucent opalescent fluid substance without visible structure (figs. 1, 2 of pl. I), closely appressed to the cell wall and bearing within it variously shaped transparent areas, the vacuoles. The interfaces of these and the cytoplasm (in other words, the so-called vacuolar membranes, **65, 66, 49, 34**) appear as thin, linear, delicate, bright bands of light. Ordinarily the vacuoles of these cells contain no visible bodies. The cytoplasmic layers between the vacuoles and the cell walls or between the vacuoles are later referred to as cytoplasmic channels.

The cytoplasm of root tip cells contains a wealth of variously shaped small bodies of diverse refractivity. The more conspicuous and the largest are irregularly rounded, usually spheroidal, starch bearing plastids. These bodies move very slowly if at all in the cytoplasmic stream, and tend to collect into groups over the nucleus, or at various other points in the cell. Spherical aggregations of starch bearing plastids are common in root cap cells. Constantly moving over and around these plastids or aggregations of plastids in the cytoplasmic stream are much smaller (0.3–0.4 μ) spherical bodies of high refractivity, the fat globules.

The nucleus usually appears as a perfectly regular sphere or spheroid in the interior of which the nucleolus appears as a spherical, faintly yellowish, finely granular, opaque structure resembling a plastid. In the cells of the apical meristem the nucleolus is large (4.0–7.0 μ) and occupies most of the nuclear volume, whereas in the elongated cells of the plerome or of the periblem the nucleolus is much smaller (1.0–1.5 μ). The nucleus shifts in the cytoplasmic stream, but this is usually perceptible only when comparative observations of its position are made over a period of hours.

Very faintly luminous bodies of diverse form move continuously across and about the nucleus, the clear vacuoles, and the aggregations of starch bearing plastids. They may be spherical structures of the same or slightly smaller diameter than the fat globules, rods, or long filaments, or threads of corresponding diameter and from 2 to 12 μ in length. These are the mitochondria. They are the most

abundant bodies and likewise the most difficult to observe within the cells of this plant.

Since this research has been directed primarily toward an analysis of the pathic state among cells influenced by a bacterial parasite, most attention has been given to those structures within the cells of living and fixed tissue which simulate the form and motion of bacteria. As the latter in many instances (**53, 90, 43, 44**) have been shown to undergo changes in morphology during their life cycle, and appear as rods, cocci, and smaller particles, all similar forms in healthy cells have been studied critically.

1. MITOCHONDRIA AND FAT GLOBULES.—In the cytoplasmic channels many tiny spherical, rod shaped, or threadlike bodies of low refractivity move amid numerous spherical forms of approximately like diameter but of much greater refractivity. During any brief period of time all these bodies usually move at a fairly uniform speed in one direction past any given point in such a channel. Within the elongated cells of the root tip, which have but thin layers of cytoplasm between the cell walls and the large central vacuole, the motion of these bodies may be clockwise about the cells when viewed in that focal plane that presents the illusion of being the cross section of the cell. It is more complex in such cells as that shown in figure 1, where connecting strands of cytoplasm traverse the vacuoles.

In such cells the bodies may be seen to come from various directions along these strands to a common point near the surface of the nucleus, where after remaining still or swaying about for a few seconds they depart serially along the same course. Along the top or bottom of the cell lumen these minute bodies of the cytoplasm often seem to follow no regular course, but swaying about for a few seconds, may suddenly move in an even regular fashion off to the wall of the cell and disappear downward from the focal field as though impelled by some definite current. Concentrating at a point, they often may move singly or in small groups off to the side and from the side across the top of the cell and down. The motion of these bodies presents every evidence of being that common to bodies themselves without motility but impelled by currents in the cytoplasmic ground substance, or hyaloplasm. The fat globules may move at the same speed as the mitochondria but generally they move

at an appreciably greater speed. The motion of these bodies indicates that there is cytoplasmic streaming in the cell. Without such ready points of reference it might not be detected.

The number of bodies included in the clear cytoplasmic ground substance is very great in root cap cells or in the elongating or unelongated cells of the periblem or plerome. The entire cytoplasm seems to be filled with them, at times scarcely leaving a place large enough for one such body unoccupied. Usually all move at a uniform speed within a stream that travels around the cell over and around the nucleus, vacuoles, and clumped masses of plastids. Within this stream each moves separately, carried by the hyaloplasm, threads bend at either end or in the middle, rod jostles over rod or spherule, so that the cytoplasm appears filled with moving bodies of various size and form. LEWITSKY (**56**) and MAXIMOV (**64**) describe a like condition for *Elodea* and *Cucurbita* hair cells.

Mitochondria vary in form in the cells of root tips. Within cells of the root cap or elongating cells of the plerome their most prominent form is a thread. Spherical forms of the same diameter are generally present with the threads, and rod forms of like diameter are plentiful. In the region of elongation of the periblem spherical and short rod forms are more common; threads are scarce. Spherical forms were the only forms seen in the chondriome of the cells of the apical meristem in fresh tissue mounts.[2] Although the mitochondria of these various cell groups generally have the form indicated, this form is decidedly not constant. Thread forms may be abundant in elongating cells of the periblem. At times thread forms may not be present in any of the root tip cells but only rod and minute spherical forms may occur. While thread and rod forms may predominate in cells of the root cap at the beginning of an observation, only minute spherical forms may be visible some hours later.

Root tip cells fixed and stained by the methods of REGAUD, BENDA, or NEMEC in general reveal the same forms repeatedly observed in fresh tissue cells. In fixed root tips, however, cells of the root cap generally are devoid of thread forms even though in other regions the elements of the chondriome as seen in fresh cells may

[2] In none of the fresh material examined was the detail of the chondriome adequately clear in these particular cells, owing perhaps to the density of the cytoplasm.

be differentiated clearly. Moreover in fixed material the cells of the apical meristem frequently contain numerous thread forms, whereas in fresh cells only spherical or short rod forms have been seen. It is also evident from repeated study of such fixed and stained material, mounted in water and observed with the water immersion objective of relatively high magnifying power, that many elements of the chondriome remain unstained and are readily overlooked if at all visible when such sections are mounted in balsam. For these reasons the observations made upon fresh tissue sections are considered to be more critical in many respects than those made on material fixed, stained, and mounted in balsam after the standard methods generally employed in research upon the chondriome.

2. PLASTIDS.—Cells of root tips in addition to mitochondria and fat globules contain another order of cytoplasmic inclusions which are generally of greater size and of varying form. These are the plastids. When devoid of starch they are of the same order of refractivity as the mitochondria, and are as difficult to see. They are spherical when seen in fresh tissue mounts of apical meristem cells. In fixed tissues they appear as rodlike or spherical forms in the cells of this region. In the elongating cells of the periblem they are spherical, rod shaped, or of bacteroid form (fig. 1). They vary upward in size from that of mitochondria. Farther back from the root tip they become larger, are generally spherical, and attain a maximum size of 5 μ. In the elongating cells of the plerome their form varies from threadlike to rodlike, or from bacteroid to spherical forms distended by starch inclusions (fig. 2). Until they bulge at one or either end or increase evenly throughout their length, they may not be distinguished from thread or rod shaped mitochondria.

Dumb-bell, hand-mirror, or thread forms with bleblike swellings occur in cells of both plerome and periblem in the region of elongation, but are more common in the former. Farther back from the root tip all these bodies are spherical or irregularly rounded and from 2.0 to 5.0 μ in diameter. In the cells of the root cap they appear spherical. In such cells they are usually very abundant, are generally grouped in motionless clusters, and appear to be chiefly of one size in any particular cell (2–5 μ in diameter) although there are

usually present less conspicuous forms intermediate in size between these and mitochondria.

The refractivity, mobility, and distribution of these bodies within the cytoplasm of the cells of root tips vary with the differentiation of these cells. In the cells of the apical meristem their refractivity is as that of mitochondria; they are evenly distributed in the cytoplasm; and neither they nor mitochondria have been seen to move. In the elongating cells of the periblem and plerome, their refractivity is slightly greater than that of mitochondria and they are evenly distributed throughout the cytoplasm. The motion of the larger ones, however, is considerably slower than that of mitochondria. Because of their large size and the high refractivity of the inclosed starch, they are the most conspicuous elements of the cytoplasm in the elongated cells of periblem and plerome and in the cells of the root cap. Their motion is not apparent although slight changes of position may be noted over a period of hours. Within such cells they tend to aggregate in clumps along the wall, and particularly over the nucleus. This clumping is characteristically pronounced in the cells of the root cap. The abundance, large size, and high refractivity of these starch bearing plastids at times impart to these cells a pronounced granular effect. Although these bodies may be of equal size and be present in equal numbers in the cells of other root tips, they are inconspicuous because of their low refractivity, which may be only slightly greater than that of the mitochondria. Plastids develop from structures which are indistinguishable from mitochondria in the cells of the apical meristems of root and stem of the black raspberry (fig. 3). This is the condition commonly reported for seed plants (**91, 32, 34, 39, 92, 71, 102, 85, 107**).

Changes in osmotic properties of the mounting medium may markedly alter the cell. The most pronounced visible effects are changes in mobility of the cytoplasm, volume and structure of the vacuolar system, and the form of mitochondria and plastids. Motion of fat globules varies considerably with the state of hydration of the cytoplasm. The motion of the cytoplasmic bodies is regular within the cytoplasmic stream in cells of fresh sections that are mounted for a short time in distilled water. Their brownian motion is faintly if at all perceptible at first, but changes rapidly as the cell imbibes

water. The large vacuoles within the cell may coalesce; if a single vacuole is present it distends, forming an even thinner layer of cytoplasm against the wall. The nucleus usually is seen in this layer as a flattened bulge against the wall. The mitochondria and plastids become irregularly swollen, diminish in visibility, and finally disappear from the cell. As they swell they aggregate in chains or clusters much as starch filled plastids do, and lose their mobility. The fat globules which moved so deliberately begin to oscillate in wider and ever wider arcs, until the cytoplasm seems a mass of wildly dancing particles, upon which no clear focus can be obtained. Now all regular streaming in the cytoplasm has ceased, the motion of the minute fat globules dancing chaotically becomes slower and slower, then finally stops entirely. At this time the vacuolar boundaries change. Up to this time they have been clear cut, gracefully rounded lines, marking the interface of the two fluids, cytoplasm and vacuole. Now, as though some inner pressure of the vacuole had been suddenly released, these lines become irregularly curved and wavy; the vacuole has collapsed. Within a few minutes the sharp line of demarcation disappears and the limits of the cytoplasm may be only inferred from the non-moving minute fat globules which the cytoplasmic ground substance contains. A few granular particles, perhaps precipitates of cyclic compounds formerly present in solution in the vacuolar fluid, are now seen, together with occasional starch grains in the vacuolar cavity in brownian motion. If a 7–10 per cent sucrose solution be substituted for the distilled water, the cells do not recover the appearance which cells of freshly cut sections have when mounted in isotonic sucrose solution. Changes in the osmotic pressure of mounting medium are of no avail. With the last faintly discernible motion of the cytoplasmic bodies vibrating *in situ*, the changes effected by anaesthesia (**46**) have become irreversible, and perhaps death has come at this moment to the cell. Hence, after 5 to 8 hours, no motion save that in collapsed vacuoles may be seen in cells of sections of tissue mounted in distilled water.

The same transition in type of motion of the cytoplasm is seen when toxic vital dyes such as Janus green B[3] are added to the

[3] The writer is indebted to Professor BLOOM of the department of anatomy, University of Chicago, for a sample of the highly purified Janus green B used by Dr. BENSLEY in his pioneer work with this dye.

mounting medium. The transition occurs within a few minutes at dilution 1–10,000 of Janus green B, but extends through several hours when the cells are immersed in distilled water.

All attempts to stain mitochondria in the streaming cytoplasm of living galls failed with solutions of Janus green B. The dilutions used varied from 1–10,000 to 1–2,000,000. The mitochondria remained unstained although clearly discernible in the streaming cytoplasm of cells that had been immersed 12 hours in dilution 1–2,000,000, Janus green B. Some of the mitochondria (rods, spheres, threads) were stained in the cells after the cells had died. Those stained were generally clumped in small irregular masses scattered throughout the rigid fixed (**12**) hyaloplasm. Janus green B, therefore, was not a useful vital stain that could be used effectively in this research. Similar results have been reported by others who have studied plant cells (**15, 85, 107, 1**).

B. Gall tissue cells

In the early stages of gall formation on the canes of black raspberry the actively dividing cells usually are regularly oriented, as though all divisions occurred tangentially to the radial axis of the cane. As the galls increase in size and age, tracheids appear irregularly distributed throughout the gall tissue; the cells in certain regions become heavily charged with tannin as do the ray cells in the stem; differentiation of most of the cells stops at the parenchyma level with various degrees of maturation indicated by decreasing cytoplasmic content. At the base of the gall new cells may continue to arise for a time, until cell production in this area of the gall ceases.[4]

Within the gall tissue, however, scattered whorls of densely cytoplasmic cells are evidence of continued cellular division within certain limited areas randomly distributed. The older galls are therefore aggregations of fairly regularly oriented, parenchymatous cells of varying age, and appear approximately uniform in size, equivalent to parenchyma cells of pericycle or phloem in canes not affected by the bacterium.

Wulff (**104**) and Butler (**11**) expressed the view that the gall is

[4] The base of the gall is here considered to be that innermost layer of gall cells in contact with cells of the stem which have not conspicuously changed in response to the bacterial invasion.

the resultant chiefly of pericyclic activity. From observations and a limited number of experiments it appears that the organism migrates upward from the crown of the plant through the tissues of the stem. At what time and through what tissue this migration occurs is not known at present.

The mitochondria of gall tissue cells (fig. 4) display no conspicuous changes that cannot be found in cells of unaffected plants. They usually are evenly distributed in the cytoplasm, and the motion induced by cytoplasmic streaming is the same as that in the cells of root tips. The mitochondria are present as short rods, spheres, or threads in the youngest cells of embryonic regions in gall tissue. Enlarging cells which are rich in cytoplasm and have multiple or single vacuoles usually have numerous thread forms. This is the condition of mitochondria in most gall cells. The number of mitochondrial filaments or rods is large in those cells which are rich in cytoplasm, and appears to be reduced in those gall cells which have thin sheaths of cytoplasm between their voluminous central vacuole and the cell walls. This condition is not appreciably if at all different from that found in the various cells of plants free of the disease. MILOVIDOV'S (**70**) observations on crown galls of *Pelargonium* and *Vicia faba* led him to similar conclusions with respect to the mitochondria in gall tissue cells.

Plastids in living gall tissue are diverse in size, form, and refractivity. In younger cells cylindrical forms with rounded ends are as abundant as spherical ones. Such rods measure 2–7 μ by 0.3–1.6 μ. The smaller forms, rods, threads, or spheres are of low refractivity, are moved about in the cytoplasmic currents, and usually are evenly distributed within the cytoplasm of the cell. The large rods and spheroidal forms with a diameter that exceeds 2 μ are shifted about very slowly, if at all, and tend to aggregate in groups along the wall in the peripheral cytoplasm and especially over the nucleus. The bacteroid form (that is, the rod form with a bulge or two along its length) occurs fairly frequently. These plastids are the most conspicuous bodies and may occupy most of the volume of the cytoplasm in older cells, particularly in galls induced on plants in the greenhouse in late autumn or early winter. Their large size, abundance, and high refractivity due to large volumes of included starch make them conspicuous.

Fat globules are present in all living gall cells. In the younger cells they are slightly larger and more refractive than the spherical mitochondria. In older cells their size and number increase markedly. This is also true of the ray parenchyma and corresponding cells of the stem. The fat globules usually are evenly distributed throughout the cytoplasm of these cells. In fixed preparations they may stain black with haematoxylin if they are preserved. Usually they are removed in the process of dehydration and clearing.

It is apparent from these observations that the mass of gall tissue is initially composed of cells in every respect comparable with analogous cells of meristematic regions of plants unaffected by cane gall. There is little evidence that they are pathic. Later changes occur in these cells, either as a result of subsequent direct or indirect action by the bacteria or lack of healthy correlation (**57, 58**) with the rest of the plant. The locus and movement of the bacteria in the gall tissue are first described, however, since these degenerative changes of gall tissue cells are conditioned by the locus and movement of the bacteria within the tissue. Before these changes are taken up, the differentiation of bacteria, plastids, and mitochondria in cane gall tissue will be discussed.

C. Micro isolation and staining

Only spherical bodies of high refractivity and of varying diameter are visible in the cells of fresh sections of cane gall tissue mounted for some time in hypotonic solution such as water. These bodies move incessantly in the cytoplasm of the living cells. The nuclei and vacuoles may be clearly discernible in the cells of such sections, as may also starch grains if present; but aside from these bodies the cells appear to be devoid of structure. The spherical bodies seen usually are 0.2–0.5 μ in diameter and have all the properties and give all the microchemical reactions characteristic of fat globules. As a result of diminished refractivity coincident with characteristic swelling in hypotonic solution, the mitochondria and non-starch bearing plastids are not visible within the cells of gall sections mounted in this way.

Cane gall bacteria, invariably present in gall tissue, are readily differentiated from the elements of the cytoplasm under such conditions. The individual thalli, uniformly short rods with rounded

ends, usually are seen in masses. Their refractivity generally is slightly lower or equal to that of fat globules (fig. 5). They appear opalescent to faintly yellowish, their surface membranes appear as perfectly regular delicate lines, and within the thalli there is no visible structure under the conditions and with the equipment named. The individual rods constituting these masses generally are regularly arranged end to end in parallel rows. They are motionless and at times appear to be imbedded in a slimy sheath. Those thalli which are seen within degenerate cells generally appear as single or bifurcated rods, slowly oscillating in brownian motion in the cell fluid.

Since mitochondria and plastids of varying size and form are visible within thin sections of fresh gall tissue mounted in isotonic sucrose solution, and since many of these units of the chondriome of the black raspberry under these conditions are like bacteria in form, size, and refractivity (fig. 4), the identity and differentiation of the bacteria from these elements of the chondriome is difficult; the picture is confusing.

Experimentally some evidence has been obtained by micro dissection as to the non-bacterial nature of these rod shaped bodies of bacterial proportion seen in living cells of gall tissue when mounted in isotonic sucrose solution. Using an aseptic technique throughout, these bodies were removed with micro pipettes and placed singly or in numbers within drops of sterile media suitable for the growth of bacteria. Coverslips bearing the hanging drops were sealed with vaseline over depressions in deep well slides containing approximately one-tenth of 1 cc. of the same medium as that in the hanging drop. These drops were incubated six days at 26° C., and were then discarded if no growth appeared. Those which produced growth were plated and the colonies which appeared were inoculated into black raspberry. The ability to induce cane galls subsequent to inoculation on one-year-old black raspberry canes was the criterion used for determining the presence of pathogenic bacterial colonies. A CHAMBERS' micromanipulator (**13**) and the technique described by WRIGHT and MCCOY (**103**) were used.

Seventeen of 21 attempted cultures of these cytoplasmic rods were negative, and from four, yellow colonies developed. As a check

on the effectiveness of this method, 23 attempts were made to isolate and culture the masses of bacterial rods found in pockets within gall tissue. Thirteen of these cultures produced no growth, eight produced cultures of yellow Schizomycetes, and two produced cultures of cane gall bacteria. Because the bacteria were successfully cultured in only 9 per cent of the isolations made from bacterial pockets, the negative results obtained in culturing the rod shaped bodies of bacterial proportion isolated from living gall cells are considered merely suggestive, not convincing.

The appearance which these rods have in culture fluids when isolated from the cytoplasm of gall cells is not characteristic of bacterial rods. The latter, when isolated from the bacterial masses of the gall tissue, habitually have a perfectly regular clear-cut outline, and a homogeneous opalescent interior which is without visible structure. The rods taken from the cytoplasm of living gall cells, when isolated in yeast broth culture medium (**88**) containing 1 per cent of glucose, had a pronounced granular appearance and were neither as regular nor as clear-cut in outline as bacterial rods[5] isolated in like manner from bacterial cavities in the gall tissue, or as these same cytoplasmic rods when observed in the cytoplasm of living cells.

A detailed study was made of fixing and staining methods to find a procedure or combination of procedures whereby bacteria might be positively differentiated from the elements of the cytoplasm with which they are easily confused. In preparations of cane gall tissue fixed in ERLICKI'S hardening fluid and stained in haematoxylin, plastids and bacteria usually are stained alike a brilliant deep blue, in striking contrast to the orange cell walls and faintly gray cytoplasm. Fat globules and mitochondria retain the stain poorly if at all, and appear light gray or are not visible. The form and size of the bacteria aggregated in the usual masses or strands are constant. The plastids are of varying size and form and are scattered throughout the cytoplasm or aggregated in chainlike or other groups at various points within the cytoplasm. In many cells their dominant

[5] These rods observed in isolated drops of culture fluid after removal from the cytoplasm probably were rodlike granules of starch remaining after spontaneous degeneration of the isolated plastids.

form is rodlike and in size and staining they are the exact counterparts of bacteria. Only an occasional transparent unstained starch granule within some of these rods, as well as their apparent identity to like forms seen in cells of healthy root tips, indicate their plastid nature.

Tissue fixed in the fluids of BENDA, REGAUD, NEMEC, or ERLICKI and stained with haematoxylin may give good fixation but no differentiation of bacteria and plastids. Cane gall tissue fixed in NEMEC'S second fluid or in certain variations of the BENDA fluids, and stained in Haidenhain's iron-alum haematoxylin, at times shows excellent fixation and brilliant elective staining of the bacteria. The mitochondria and plastids may be readily destained, leaving an intense blue only in the bacteria. Plastids and bacteria retain the stain in like degree in other fixations with these same fluids. Differentiation between rodlike plastids and bacteria is therefore not possible with this stain, and the results are confusing. Because of gross distortion in form and size of mitochondria, plastids, and bacteria, other fixations with the NEMEC or REGAUD fluids may be worthless. When used alone, therefore, this method might lead to the conclusion that an intracellular parasite characterizes gall tissue.

Excellent differentiation of the bacteria from all elements of the cytoplasm in cells may be secured by fixing the tissue in BENDA'S chromic osmic fluid, hardening in ERLICKI'S fluid, and staining in haematoxylin. Bacteria, plastids, and mitochondria are well fixed. The mitochondria and plastids may be readily destained to a faint gray, however, leaving an intense blue only in the bacteria. The plastids and bacteria, which may be alike in form, are differentiated sharply by the faint gray of the former and the intense blue of the latter.

II. The parasite and its relation to host cells

A. IDENTIFICATION OF BACTERIA OBSERVED IN CANE GALL TISSUE

Since most bacteria probably would stain alike if present in cane gall tissue, and since bacteria other than the causal organism might bring about some of the cellular effects observed, particularly the disintegration of the tissue later described, it was considered nec-

essary to establish the identity of the bacterium associated with the cellular and tissue changes noted in the galls. Isolations were made from many galls of various ages induced by pure culture inoculation of plants in the greenhouse or garden, or following natural infection on black raspberry plants in Michigan or Indiana plantations. Fragments of gall tissue, approximately 6–8 cubic mm. in volume, were removed aseptically from various portions within the surface or from the surface of the galls,[6] and transferred to sterile petri dishes containing 2 cc. of sterile water.

The bits of tissue were then macerated, loop dilutions were made to other plates, and from these in turn loop dilutions were made to a third set of petri dishes. All plates were poured with 2 per cent potato dextrose or 1 per cent yeast glucose agar and incubated for ten days at room temperature. Usually 100 or more colonies were obtained, and of one kind, when the isolation was made from the interior or surface of galls that were not more than 25 days old. This type of colony appeared in the potato dextrose poured plates after the fifth day, and after several additional days of incubation was a convex white butyrous small colony 1.5–3.0 mm. in diameter. Its ability to induce cane galls when inoculated into black raspberry was taken as criterion for identification as the cane gall bacterium. Older galls usually but not always yielded increasing numbers of rapidly growing colonies of yellow Schizomycetes and a decrease in number of colonies of cane gall bacteria. Plates poured from the tissue of galls more than two months in age generally yielded only yellow colonies. However, in view of the enormously greater number of colonies of cane gall bacteria isolated in the plates poured from all galls which externally were free of decay, and from gall tissue which microscopically showed the characteristically marked internal tissue degeneration described later, it was inferred that the bacteria isolated from the tissue are the incitants of the cellular changes which characterize cane gall.

That all question as to the identity of the bacteria observed in the tissue might be removed, galls were induced under aseptic con-

[6] The surface of a gall is taken to be that layer of living or dead cells in contact with the environment. Cane galls have in no case been found to have any organized group of cells comparable with a periderm or a root cap.

ditions within glass cylinders, using the technique described by BANFIELD (4). Three weeks after inoculation, fresh white frosty overgrowths were present within these cylinders. On first-year black raspberry canes they extended 0.5 cm. radially from the stem at the points of inoculation. The cylinders were filled with sterile water for ten minutes prior to the removal of the galls for microscopic study. This water was diluted 1–1000 with sterile water, 1 cc. of the dilution was placed in each of several petri dishes, the dishes were poured with 2 per cent potato dextrose agar, and incubated at 24° C. for seven days. Only the cane gall bacterium appeared in the plates. The number of bacteria present per cc. of wash water was 48,300 as determined by the plate count. Cane gall bacteria were the only organisms that appeared in agar poured plates in which sections of these galls were macerated.

Microscopic examination of the tissue of these galls, living or fixed, revealed that they differed in no important way from galls induced underground or aerially in the greenhouse, field, or garden.

B. LOCUS AND MIGRATION OF BACTERIA IN TISSUES OF CANE GALLS

The bacteria migrate through the tissues in zoogleal strands which separate the cellulose walls of gall tissue cells by dissolution of the middle lamellae. Their grouping in chains or in masses is characteristic; they do not occur singly. At the tips of the advancing strands there frequently is only a single bacterial rod between the parting cell walls. More commonly the advancing tip is lobed and contains a number of bacteria. Behind such lobate tips the bacteria generally are regularly oriented end to end and evenly spaced, forming a strand or column of multiple parallel rows. The advancing tip of the strand may be decidedly broad, the inclosed bacteria irregularly oriented, and spaced in the tissues of galls which are subjected to a shower for twelve or more hours prior to fixation of the tissue.

Bacterial pockets commonly occur in the meristematic areas of cane gall tissue. They result from successive lysis and collapse of cells in contact with bacterial strands, accompanied by increase in the mass of bacteria. The cells in contact with the expanding bacterial mass are lysed and irregularly compressed inward, thereby im-

parting an irregular outline to the bacterial mass. These pockets become numerous as the galls increase in age.

Extensive intercellular penetration by the bacteria may induce maceration and complete lysis of areas of cells, thereby creating cavities of considerable magnitude in the tissue. The areas affected are chiefly those cells in older galls which are at a distance from the bacterial pockets and outside the meristematic zones that encompass bacterial pockets. Individual cells or groups of cells in such areas may be separated from the tissue by complete dissolution of the lamellae and be engulfed by the bacterial mass.

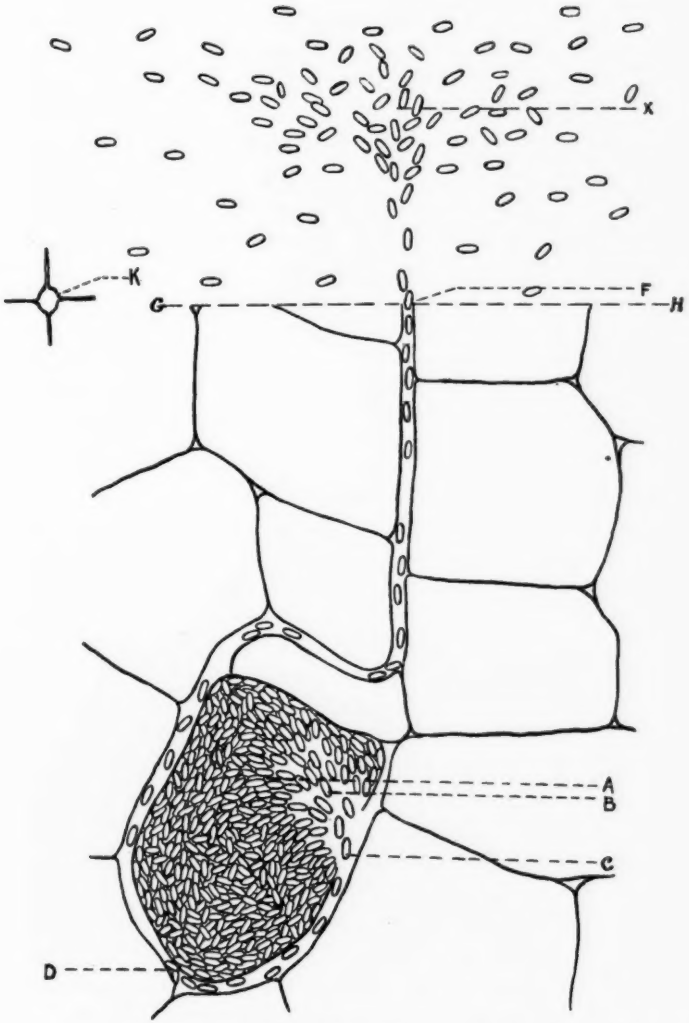
Within the cells of these areas, swarms of bacterial rods are frequently seen oscillating in brownian motion. These protoplasts are degenerate, however, and only fragments such as fat globules remain within their partially hydrolyzed walls. Occasionally small compact groups of bacteria have been observed within protoplasts which show no pronounced disorganization of cytoplasm or nucleus. Usually, however, penetration of the cell walls occurs only after the protoplast has been extensively lysed and disorganized. Cane gall bacteria are therefore primarily intercellular.

Cane gall bacteria found in strands and masses in gall tissue commonly are inclosed in a gelatinous matrix which in fresh tissue appears as a translucent clear material in which the bacteria are without motion. This imbedding matrix is stained a clear faint yellow in tissue fixed in the fluids of NEMEC, BENDA, REGAUD, or ERLICKI, and stained with haematoxylin and orange G. The bacterial rods in sharp contrast are stained an intense blue. This matrix usually is viscous as determined with micro dissection needles, and individual rods imbedded in it are not readily removable. The bacteria of these masses frequently have been observed in brownian motion in other sections of fresh tissue. They are then easily removable by micro pipettes from these masses. Under such conditions it is obvious that the viscosity of the imbedding material of the so-called zoogleum has been markedly reduced and varies but little from that of the dilute sucrose solution of the mounting medium.

The bacteria appear to be discharged from the tunnels and pockets in which they exist in the gall tissue on to the surface of the gall prior to its disintegration. Similar observations on fire blight

cankers have been reported by NIXON (**80**). This discharge occurs after liquefaction of the gelatinous material in which the bacteria are imbedded. Evidence of discharge is of four types: (1) many cavities or pockets obviously created by the bacteria are found in cane gall tissue, but at the time of observation contain few or no bacteria; (2) bacteria are always found on the surface of both cane and crown galls of black raspberry; (3) experimental evidence of the continuous escape of the bacteria from crown galls has been presented by ROBINSON and WALKDEN (**89**) and by BANFIELD (**4**); and (4) the bacteria have been observed in the process of discharge via intercellular channels from cavities included in sections of fresh cane gall tissue.

Discharge of cane gall bacteria from interior pockets via intercellular channels has been observed on several occasions in sections mounted in 7.5 per cent sucrose solution. This discharge is illustrated in text figure 1. In a cavity below the sectioning plane (*GH*) a faintly discernible shifting of bacteria occurs within a dense mass of independently motionless bacterial rods, in zone *A* accompanied by a very slow motion oriented toward a focal point *B*. At a point midway between *B* and *C* this shift becomes distinct and the bacteria are oriented end to end as sketched. This orientation and the acceleration in motion that follows are much like that which occurs to driftwood in the smooth water immediately above a rapid in a river. From *C* to *F* the motion of the rods is extremely rapid but decidedly jerky, as though instantaneous stopping of the intercellular channel of discharge occurred (as at point *D*), followed by momentary release, stopping and release, etc., causing the bacteria to move at high velocity in spasmodic jerks. The general picture is similar to the discharge of spores in certain Oomycetes (**20**). The bacteria are discharged from the opening of the intercellular space at the surface of the tissue *F* (the surface formed by the plane of sectioning). The velocity of the bacterial rods is quickly lost in the zone *F* to *X*, and beyond *X* the motion is that of slow diffusion radially away from the point of discharge. The bacteria, now floating free in the mounting medium bathing the tissue, oscillate in brownian motion but otherwise their motion is not appreciable. They may become extremely numerous in the immediate area about



TEXT FIG. 1.—Discharge of cane gall bacteria as seen under the microscope from an interior cavity within a fragment of gall tissue. The bacteria are being discharged via an intercellular space formerly occupied by a zoogleum into the mounting medium (7.5% sucrose).

the point of discharge, *F*, but each remains free and there is no tendency to agglutinate into masses as is exhibited by the elements of the chondriome in autolyzing cells.

The channel through which this discharge occurs appears in cross section as at *K* in the sketch. Invaginations of the normally distended cell walls are characteristic of these channels of escape. From this, and from the like contour of cross sections of intercellular spaces occupied by bacterial strands, it appears that these channels are passages which were occupied by bacterial strands whose contained thalli have been discharged.

C. Effects of bacteria upon host tissues and cells

The gall development which the cane gall organism incites on fruiting black raspberry canes appears to be essentially wound response. The abundant cellular division by which the gall tissue is formed occurs primarily in those regions which are at a distance from the bacteria.

Hyperplasia of the pericycle and phloem ray cells induces the gall initials or bulges found beneath the phelloderm of fruiting canes in early spring.[7]

The first series of divisions leads to the formation of a sheath of cells which at first envelopes the initial foci of infection. Into this sheath the bacteria subsequently penetrate radially from the initial foci of infection. The cells surrounding these strands in turn are stimulated to division, the cross walls of the new cells being laid down parallel to the bacterial strands or masses, the source of stimulus. Lateral penetration among these cells by bacterial strands may occur and again be followed by division of the cells surrounding the penetrating strands. As the galls increase in size and age, however, hyperplasia becomes progressively less.

Cellular degeneration and necrosis are coincident with hyperplasia from the time of the first microscopic appearance of the gall in early spring. The cells in contact with the bacteria gradually undergo cytolysis, those cells beyond divide and divide, while those at a farther distance apparently are not stimulated. In the early stages

[7] Hypertrophy of cells, a common response of many plant cells to tissue invasion by the crown gall organism (**97**), does not occur in this disease on black raspberry.

of gall development, however, new cells are produced so rapidly and in such profusion that the lytic action of the bacteria on the relatively limited number of cells with which they are in contact appears insignificant. The division of cells becomes progressively more feeble, however, the bacteria more and more numerous, intercellular invasion more extensive, and ultimately tissue degeneration becomes general.

This degeneration is the result of two types of cellular disorganization and disintegration: (1) *Cytolysis* is a degeneration characterized by the progressive lysis of the plastids, mitochondria, nucleus, fat, and eventually the walls of protoplasts in contact with bacteria. The fat volume of protoplasts undergoing this type of degeneration is greatly increased. (2) *Autolysis* is a degeneration characterized initially by changes in the condition and aggregation of the chondriome to form various irregular solid masses, alveolar, granular, reticular, open three dimensional network, or perforated plate structures in the cytoplasm. In this degeneration all elements of the cell eventually lose their identity and are blended in an amorphous mass of diminishing volume, compressed between collapsing walls. It usually is accompanied by no appreciable changes in the fat volume of the protoplast. It occurs most conspicuously in masses or large areas of cells enveloped by or separated from the rest of the tissue by sheaths of cytolyzed cells and bacteria.

In the degeneration of the tissue large numbers of cells disintegrate as a result of cytolysis. The great majority of cells degenerate through autolysis, however, probably as a result of lack of healthy correlation with or complete isolation from the rest of the tissue. These degeneration phenomena are apparent upon casual microscopic observation of the gall. The direct effects of cytolysis are more conspicuous during or for a short time after periods of rain in the blossoming period of the black raspberry. At such times larger areas of the outer tissue of the white rounded gall masses become butyrous to the touch; the tissues offer no resistance to slight pressure and readily rub away. Extensive intercellular invasion of bacteria and dissolution of lamellae have macerated these areas of cells. A few days later the galls appear extensively pitted. They undergo no conspicuous changes in color as a result of this type of

degeneration, and remain white or have a slight cream color and a water soaked appearance. The effects of autolysis are more apparent later. The gall tissue turns yellowish, then brown, and shrivels. Galls induced by inoculation undergo like changes.

The progressive lysis of cells in contact with bacteria generally appears to proceed in the following manner. Starch is rarely present in such cells. The plastids are reduced to elongated or threadlike forms which after a time, if still present, are indistinguishable from the small rodlike and spherical mitochondria (fig. 5). Mitochondrial rod and thread forms are reduced in number, while the small spherical forms increase. Fat becomes conspicuously more abundant in the cells.[8]

The nucleus is reduced in size and after a time displays a faintly granular or reticular internal structure. Streaming of the cytoplasm ceases, for a time the cytoplasmic bodies vibrate in brownian motion, then all motion ceases. The mitochondria disappear, fat globules and the nucleus remain (fig. 6). The granular structure of the nucleus is accentuated, and the nucleolus is reduced in size proportionate to the reduction in volume of the nucleus. The outline of the nucleus becomes irregular and finally the nucleus disappears. Fat is usually the last substance to disappear from such cells. Many cells are reduced in this way, leaving merely their walls. Along the borders of bacterial pockets or strands, the walls of these cytolyzed cells collapse and are mingled as débris among the bacterial mass. It has not been possible, however, to follow any one cell through this entire seqùence of changes.

The cellulose walls of these cells in the majority of instances are not dissolved by the bacteria. They may retain their usual position during or subsequent to the lysis of the protoplast; they may be slightly compressed by the expanding mass of bacteria; or they may collapse entirely after complete lysis of the protoplast, so that their inner surfaces touch. Small areas of the wall may be dissolved by bacterial masses which then may occupy the entire cell lumen. In

[8] This rise in fat volume is common in cellular degeneration induced on a wide range of plant and animal cells by fungi and bacteria (**21–29**), exposure to radium emanations, x-rays or ultraviolet light (**72, 75, 76, 27**), various lipoid solvents (**6, 7, 73, 74**), heat (**54, 84**), osmotic changes (**34, 8, 9**), poisons (**94**), inanition (**79**).

areas of tissue maceration, however, the lytic action of the bacteria may include complete lysis of the walls of many cells and partial lysis of cells at the periphery of the area of completed lysis. The end result may then be an empty irregular gap in the tissue after the bacteria have been released from the area. Occasionally advancing bacterial strands appear to pass through cellulose walls but as yet no convincing demonstration of this has been observed.

Usually cytolysis occurs relatively slowly and is observable more commonly in cells which have but a portion of their wall in contact with the bacteria, although it may occur in those areas macerated by the bacteria. In the latter instance it may be effected in like manner but much more rapidly. Usually within the cells of areas extensively penetrated by bacteria autolytic changes occur before cytolysis is completed, and the tissue of these areas degenerates as a result of both types of cellular disintegration. There is a pronounced rise in fat volume in these cells coincident with extensive bacterial penetration, and the plastids generally are reduced in size or disappear (fig. 5). At this stage the remaining elements of the chondriome may fuse into a network (fig. 8), reticulum (figs. 7, 8), or agglutinated mass (fig. 9). The vacuoles and nuclei in such cases appear relatively unchanged. In more pronounced degeneration of this type the vacuoles disappear and all elements of the protoplast lose their identity in an amorphous mass of débris occupying a small space within the collapsing walls.

Mitochondria, plastids, and fat move freely about over and around one another in the streaming cytoplasm of non-pathic cells (fig. 4). As the cells undergo autolysis, spatial isolation of these bodies generally is lost. The elongated plastids and mitochondria of such cells may display a pronounced tendency to adhere wherever they touch each other. For a time this union may be transitory, and the mitochondria join, break away, then join with others. The ends of the same mitochondrial filament may fuse, producing a ring, or several may fuse in various ways, the result being a small open network. These rings and small networks may for a time be carried about in the cytoplasmic stream. Other mitochondrial filaments touch and fuse, the process continues, and the network enlarges by addition of more and more of the free mitochondria of the cell. Free

filaments coming into contact with each other or with the network of filaments are held. The filament may then break away, move about in the cytoplasm, touch another filament and again be held momentarily or permanently. Eventually all the mitochondria may become fused into this network. The network may be in one plane but generally is three dimensional, and often is generally distributed throughout the major area occupied by the cell (fig. 8).

Small bacteria have frequently been seen to move through such cut cells under and over the filaments of the network in water mounts of fixed sections observed with the water immersion objective. This would not be possible in a *multiple* vacuolate condition such as that implied repeatedly by DUFRENOY (**21–29**; in particular **21**, pls. IIIa, VI, VII).

Whether production of the network structure proceeds from start to finish in this fashion or is subsequent to and a progressive stage of the plastid reticular fusion elsewhere described is not clear. The type of fusion here described is common to cells wherein fusion of the chondriome has already attained considerable proportion and the free mitochondrial filaments are conspicuously reduced in number. Free spatially isolated non-starch bearing plastids, such as are numerous in non-pathic cells, generally are not present in these cells. This absence of plastids, and the observation that the plastid reticulum may transform into a network structure, suggest that reticular fusion may be an initial stage in the formation of network structures. The fusion of limited numbers of mitochondria or plastids has been described repeatedly (**6, 7, 69, 42, 28**). CHAMBERS (**12**) has described the mitochondria of an Orthopteran germ cell clearly stainable by Janus green B as forming a voluminous granular network. Fusion of the elements of the chondriome to form networks has been reported to occur in tissue culture of chick embryo heart by LEWIS and LEWIS (**54**, figs. 5, 6, 8; **55**, fig. D, pl. III). DUFRENOY (**21, 22**) and BEAUVERIE (**7**) have reported an increase in the mitochondrial complement of various types of plant cells prior to their disintegration.

There appear to be currents in the cytoplasm during the formation of the network. These currents carry the mitochondrial filaments from place to place prior to their more permanent fusion.

Doubtless this movement of the cytoplasmic ground substance induces the continuous swaying of the filaments of the reticulum or network for a time, even after all mitochondria of the cell have become fused into the network. Fat globules also move about in cells in which mitochondrial networks are forming. They may lie free and vibrate in brownian motion between the swaying network strands. Frequently they appear to adhere to the strands before or after the mobility of the cytoplasm has been lost.

The mitochondrial network may undergo condensation or it may break up into a granular débris. Smaller condensed masses of heavier network strands and plate structure of high refractivity are frequently observed in cells wherein all cytoplasmic bodies have lost their identity. Mitochondrial networks have been observed to contract during the course of observation, and by a continuation of the fusion process to give rise to similar but condensed and knotted networks, perforated plate, or massive strand structures. The latter are decidedly vacuolate in appearance at times owing to open areas in the original network which become partially inclosed by membrane-like sheaths formed by lateral fusion of mitochondria and plastid filaments. Condensation of plastids and mitochondria wherein no initial network is formed produces somewhat similar masses of material of heightened visibility (fig. 9).

Fragmentation of mitochondrial networks has been observed. It occurred gradually in networks in fresh tissue that was studied over a period of hours and suddenly when distilled water was added to the sucrose solution in which the sections were mounted. In the first instance networks were clearly visible in numerous cells of a particular microscopic field during the two hours immediately following sectioning and mounting of the tissue. The networks observed in two cells of this field were sketched. Four hours after mounting, condensation had occurred in the network of one of these cells. The strands of the other were of markedly diminished visibility, and at the end of the following hour only granular particles and clear-cut fat globules could be seen. The network had apparently disintegrated. The addition of distilled water to the sucrose mounting fluid bathing another section of fresh tissue caused the swaying network in several cells to break up within a few moments to granular

débris in which only a few mitochondrial rings, short rods, and network fragments could be readily identified. Associated with areas of cells in which clear-cut networks abound, there are usually other cells in which mitochondrial fragments (rods, spherical forms, occasional threads with their long axes parallel to each other and closely appressed) lie massed together and irregularly distributed in the cells.

This is readily observable with a water immersion objective in fresh or fixed material (fig. 9). It is difficult to detect in sections fixed, stained, and mounted in balsam because of irregular staining or no staining at all. When stained the individual elements are not resolvable and blend together in an opaque mass, unstained; the difference in refractivity between them and balsam is not great enough to make them visible. PARAT (**81**) points out that the intense staining or chromatophile area of degenerate cells where strand structure is apparent are areas where mitochondria and vacuoles are in intimate contact and where lipoids are particularly abundant.

The cells in which fragmentation of the network has occurred generally are found nearer to the surface of the gall than are the cells in which no fragmentation has occurred. Only granular débris is found in cells still nearer to the surface or periphery of the gall. Even this granular appearance is lost and nothing remains but a spongy homogeneous mass in collapsed cells on the surface of such areas of the gall. In progressive degeneration of cells observed in fresh and fixed tissue, the granular débris present at certain stages is very probably of mitochondrial and plastid origin.

Mitochondria may not display any tendency to fuse prior to the moment of death. With the cessation of cytoplasmic movement they are held *in situ* in the rigid cytoplasm and remain for a time spatially isolated. Their refractivity is not diminished. Lying in slightly different planes curved about the margins of numerous vacuoles, and overlapping, they may produce symmetrical patterns which may impart a superficial alveolar structure to the cytoplasm. Careful observation, however, reveals this apparent structure to be the result of numerous variously curved mitochondrial threads, isolated from one another in the clear cytoplasmic ground substance by very slight distances.

Radial strand structures are produced in certain areas of degenerating cells. This structure, composed of irregular strands of varying mass and diameter, probably arises by subsequent hydration of the rigid clear cytoplasmic ground substance and fusion of mitochondria and plastids that are spatially isolated at the moment of cellular death. In many of the cells of certain areas studied in fresh sections in which this strand structure was prominent, mitochondria and plastids were observed to be spatially isolated in the rigid hyaloplasm. In adjoining cells mitochondria and plastids were seen to lie close together and were tightly interwoven to form strands (fig. 9). In others the elements that made up this particular type of strand were apparently fused and usually gave no indication as to the origin of the strands. Starch grains are occasionally included in these strands, however, and the position of mitochondria and plastids in adjoining cells indicates that the strands are products of these elements of the cytoplasm. A radial strand structure, the strands of which are perfectly regular in outline and of uniform diameter throughout their respective lengths, may also result from the plastid mitochondrial reticular fusions which occur prior to the moment of cell death.

Mitochondria may display no tendency to fuse before or after death of the cell, and lose their identity with the other cytoplasmic structures in granular débris. A few small spherical or rodlike forms of very low refractivity may still be identified as of mitochondrial nature in such cells in fresh tissue. In material fixed in the fluids of BENDA, ERLICKI, NEMEC, or REGAUD and stained with iron-alum haematoxylin, the granular appearance is accentuated by the uneven staining of the various granular particles, among which occasional rodlike, short thread, or spherical forms may still be readily identified as of a plastid or mitochondrial nature. All cellular elements in such cells lose their identity after a time and are resolved into the doughy compact mass of irregularly folded homogeneous material lying between the collapsing walls of degenerate cells. This material in fresh sections takes a faint red stain with Sudan III and browns somewhat with osmic acid. It stains intensely with haematoxylin, after the fixations enumerated in an earlier section. Granular structure in the cytoplasm is not found in cells wherein the mitochondria

have fused to form various reticular strand, network, or mass structures. These condensation structures do not stain with osmic acid or Sudan III in fresh tissue. The larger strands and masses and certain of the reticuli stain intensely in haematoxylin after fixation as described. The networks and isolated filaments are more difficult to stain, and are apt to stain irregularly or not at all with the usual staining schedule for plastids and mitochondria.

Plastids may aggregate end to end in chains, and by fusion their ends may become resolved into a reticulum in autolyzing cells (fig. 7). Such reticuli and the various stages in their formation have been observed in both fixed and fresh cells. In the initial stages bodies clearly of plastid nature are seen in chainlike aggregations. The limits of the individuals between the ends of the chain later are not resolvable, and the entire chain oscillates slowly as a unit in the cytoplasmic stream. In other cells the reticulum is longer and more mobile. The irregular diameter of this reticulum throughout its length, its refractivity and staining properties, indicate the origin of the reticulum. The progressive decrease in the number of free plastids in the cytoplasm appears to vary directly as the increase in size of this reticulum. Finally few or no free plastids remain. Mitochondrial filaments may fuse into a reticulum or network structure prior to, subsequent to, or simultaneously with the fusion of plastids into a strand structure.

Bodies which appeared to be plastids have been observed in the vacuoles of cells in degenerating areas of gall tissue. These bodies were of characteristic plastid size, form, mobility, and refractivity. In one instance a dumbbell-shaped plastid-like body was observed to be extruded almost instantly from cytoplasmic ground substance into a vacuole. A number of other elongate club-shaped, dumbbell-shaped, and variously rounded irregular rodlike bodies with clear-cut regular outline were already present in the vacuole of this cell. Like bodies of usual plastid form, size, refractivity, and mobility were also present in the cytoplasm of this cell.[9]

In other cells wherein all plastids have disappeared from the

[9] Tannin globules of like and varying diameter are also present in gall cells. They are generally perfectly spherical and, unless treated with intra-vitam dyes or other reagents, are not visible under the conditions of this research.

cytoplasm, long filaments, uniform in diameter throughout their length, have been repeatedly observed in the central vacuoles. These filaments are of characteristic plastid refractivity, are extremely long, and form a profusely recurved reticulum. There may be several shorter ones but usually there is only a single long one in the vacuole. The nuclei of such cells display no conspicuous variation from that observable in the nuclei of non-pathic cells. Mitochondria are clearly visible in usual number and size in the cytoplasm of some of these cells which have reticuli in their vacuoles. Fat is unchanged. In one instance, in a cell in which such a reticulum was observed at the beginning of a three-hour observation period, the reticulum had disappeared from the vacuole at the close of the period. A network structure composed of strands of like diameter was then present at the interface of vacuole and cytoplasmic ground substance.

Plastids display a pronounced tendency to agglomerate and fuse to solid masses in autolyzing cells. The large starch bearing plastids commonly clustered about the nucleus of living cells generally fuse into a dense agglomerate mass in the autolyzing cell. Smaller groups scattered throughout the cytoplasm may also agglomerate and fuse into irregular homogeneous masses of heightened refractivity. The outlines of these masses for a time are studded with the spherical protuberances of but partially fused plastids. Eventually fusion becomes complete, and all traces of the origin of the substance composing the mass are lost as the individuals which make up the mass lose their identity in it. Starch grains are frequently observed in these masses in the initial stages of fusion, but apparently are soon digested since they are not generally found in the compact smooth masses of the end stages of fusion.

Associated with plastid fusion of this type is the mitochondrial fusion already described. Radiating from the agglomerated plastid masses, irregular strands and plates of fusing mitochondria or the strands of mitochondrial networks frequently are seen. Isolated plastids are also found in these strands. Occasionally, even after fusion has progressed to the point of obliterating the origin of these strands, the fact that plastids have formed a part of their substance is disclosed by an occasional starch grain in the strands.

Agglutination of the chondriome to form homogeneous masses of

varying pattern and size in the cytoplasm of degenerate animal tissue cells has been described by MAYER, RATHERY, and SCHAEFFER (65, 66), SCOTT (94), CHAMBERS (12); and in plant cells by BEAUVERIE (6, 7) and DUFRENOY (21, 28). DUFRENOY attributes the so-called X bodies or inclusion bodies of degenerate virus cells to this agglutination and vacuolization of plastids. SHEFFIELD and SMITH (96, 100, 95) describe similar agglutination phenomena as forming X bodies in virus cells but they are noncommittal as to the identity of the agglutinating elements.

A number of observations suggest that plastids may form part of the strands of the network seen in certain groups of autolyzing cells. The spatially isolated rod or elongated plastids, prominent particularly in mature or cytolyzing cells, are conspicuously absent in the cells wherein the network structure has been completed. The fusion of plastids into reticuli in which the identity of the substance of the recurved filaments is lost, and the transformation of this into a network structure (as already described), are also evidences of plastid inclusion in the substance of the networks described.

Plastids and mitochondria may undergo vacuolization and give rise to an aveolar structure in the cytoplasm of autolyzing cells. The first changes noted in sections of fresh tissue are the cessation of cytoplasmic streaming and an aggregation of granular material in the cytoplasm. In the functional cell the mitochondria move freely in the cytoplasmic stream and are clear-cut in outline. In these cells they are indistinct, aggregated, and motionless. They appear to be swollen. The plastids appear irregularly spheroidal, larger than in non-pathic cells, and give no starch reaction. They are usually aggregated over the nucleus, but may aggregate in smaller groups or lie singly in the cytoplasm along the walls. Because of the marked reduction of visibility of the mitochondria and plastids in such cells, the more satisfactory observations have been made on material fixed in ERLICKI's fluid and stained with iron-alum haematoxylin.

The plastids are stained an intense blue black; the mitochondria are generally unstained in cells of gall tissue fixed in ERLICKI's fluid and stained in haematoxylin. In contrast, the swollen plastids and mitochondria of autolyzing cells in these same preparations are stained a faint clear blue. The plastids vary in size upward to four

or five times their usual diameter. Mitochondrial threads occasionally present appear irregularly swollen, as is usual with mitochondrial threads in the cut cells of fresh root tip sections. The spherical form, however, aggregated into compact masses, is characteristic of these autolyzing cells. These small spheres, all of approximately the same diameter, may be aggregated in groups apart from plastids or they may be intermingled with plastid groups. The plastids are spatially isolated from each other as are the mitochondria in non-pathic cells. They are aggregated in clumps scattered about the cell, or more generally they are aggregated over the nucleus of autolyzing cells. Swollen plastids and mitochondria may also be aggregated in chains or arranged in strands radiating from the nucleus to the walls, creating symmetrical patterns of beautiful design. In many instances these aggregations of swollen mitochondria and plastids impart a distinctive foam structure to the cytoplasm.[10]

Where degeneration has progressed further, an irregular very faintly staining membrane-like film of foamlike structure, irregularly contracted, remains. Only scattered wisps of the plastid material remain in others. The apparently rapid reduction in the amount of plastid and mitochondrial substance suggests that the marked increase in surface induced by this vacuolization brings about lysis of the chondriome much more rapidly than it is accomplished subsequent to the plastid mitochondrial condensations described elsewhere.

Fat does not become conspicuously augmented in volume in autolyzing cells. This is one of the conspicuous differences between the cytolysis and autolysis described. Fat is usually present in autolyzing cells in the same sized globules and in like frequency as in functional cells. Increase in age of gall or normal stem tissue, as stated elsewhere, is accompanied by increase in the quantity of fat found in the cell. Occasionally in the débris of autolyzing cells wherein all normal structure has been lost a few large fat globules 2–5 μ in diameter are found. These stain red with Sudan III and blacken in osmic acid. Usually no fat globules are detectable in the

[10] Vacuolization of the chondriome has been reported frequently in degenerate animal and plant tissue cells (**5, 34, 35, 39, 42, 6, 7, 9, 73, 74, 22, 24, 26, 29, 69, 93**).

doughy or spongy débris that remains between the collapsing walls of disintegrating cells. This material stains intensely with haematoxylin after fixation in the usual mitochondrial fluids. It also takes a faint red stain with Sudan III and browns with osmic acid fumes. This may be indication of the presence of fat in the cellular débris, but like all other elements of the protoplast it apparently loses its individuality and is blended into the amorphous mass that remains between the collapsing walls of disintegrating tissue.

From the foregoing description of the cytoplasm of functional and autolyzing cells of the raspberry, it appears that such structure as is visible in these cells under the compound microscope is the structure imparted to the degenerating cytoplasm by the various states and arrangements of its included bodies, namely, mitochondria, plastids, and fat. The cytoplasmic ground substance in fresh tissue is a translucent viscous fluid, which after fixation in the fluids of BENDA, NEMEC, ERLICKI, or REGAUD has a very finely granular appearance (fig. 3). The reticular, alveolar, radial strand structure, or network structure observed in autolyzing cells in fresh or fixed tissue, is of mitochondrial and plastid material. The coarse granular structure present in the cytoplasm of certain autolyzing cells, or in non-pathic cells that have been treated with ethyl alcohol (coagulation), is also due to distortion and degenerative changes in the mitochondria and plastids of the cytoplasm.

III. The crown gall controversy

The preceding observations have some bearing on the controversy concerning the position of crown gall bacteria in the tissue of its host plants and the structure of cytoplasm.

SMITH in 1912 (**97**) presented the evidence which led him to believe that the bacterium is an intracellular parasite. He observed numerous granular bodies, many of which were rodlike and seemingly of bacterial proportion, in the cells of gall tissue that had been treated with gold chloride and formaldehyde. In 1920 (**98**), however, he admitted that these bodies probably were mitochondria. In 1925 (**99**), after additional evidence had been presented pro and con, he last expressed his views on the subject in an address before the French Academy of Sciences, and said, "La localisation (-inter ou intra cellulaire-) n'est pas connu."

RIKER (87) and also ROBINSON and WALKDEN (89) took issue with SMITH and demonstrated that cell division and multiplication of gall tissue cells took place in sheaths about what apparently were masses of bacteria in crown gall tissues of tomato, tobacco, and sunflower. They concluded therefore that the position of the organism was intercellular. This view was reaffirmed by MAGROU (61, 62, 63) upon the basis of analogous studies on crown galls of *Pelargonium*, and again by BERRIDGE (10) who studied crown galls induced on sweet pea and tomato. More convincing evidence of the correctness of this conclusion was presented by HILL (47), who demonstrated and published unequivocal drawings showing the intercellular position of crown gall bacteria in early stages of infection in tomato.

Exception to the intercellular interpretation of the locus of crown gall bacteria within the tissues of its host plants was taken by PINOY (83) and by NEMEC (77, 78). PINOY reported bacteria-like bodies on the surface and within certain tannin bearing cells of crown galls on *Pelargonium*. NEMEC (78), in a report on cytological investigations of crown galls occurring naturally on plum nursery stock, said, "Es enthalten alle Gewebearten der von mir untersuchten Tumoren innerhalb der Zellen Bakterien oder bakterienähnliche Gebilde." He inferred that these bacteria-like bodies, which he observed within the cells of plum galls that had been fixed in NEMEC's first or second fluids, were crown gall bacteria because of (1) their uniform size, (2) their tendency to collect in bacteria-like groups in the cytoplasm of gall tissue cells, and (3) the specific differences between these bodies and mitochondria as to form, size, and selective staining properties.

On the basis of the studies herein reported, it is concluded that the bacteria-like bodies observed by SMITH and later by PINOY and NEMEC within tissue cells of crown galls were normal elements of the chondriome of the cells and not bacteria as they at one time believed, for the following reasons.

1. The plastids of most seed plants at certain stages in cell maturation are similar to if not identical in size and form with phytopathogenic bacteria. The tendency of plastids in the cells of seed plants to aggregate in bacteria-like groups within the cytoplasm also is general.

2. Equivalence in size, form, and staining reaction of bacteria and

plastids at certain stages of cellular maturation in cane gall and crown gall tissues on the black raspberry has been demonstrated.

3. Plastids commonly are more readily fixed and have a greater affinity for stains (**92, 71, 68, 107**) than have mitochondria. The destruction of mitochondria and the resistance exhibited by plastids to fluids containing acetic acid have been shown by GUILLIERMOND (**32, 34**) and COWDRY (**14**). The writer has found this to be true of plastids in cane gall and crown gall tissue after fixation in fluids containing acetic acid such as the formalin acetic alcohol commonly used by SMITH (**97**). Selective staining of plastids in cane gall or crown gall tissue on the black raspberry is readily attained after fixation in the fluids of NEMEC, ERLICKI, and frequently after fixation in BENDA'S fluid.

4. The bacteria in crown gall tissue of the black raspberry are frequently decidedly limited in number and in many sections of such galls none may be found. The probability of not observing them and of mistaking selectively stained elements of the chondriome for the bacteria is considerable.

5. It has been demonstrated in this research that cane gall bacteria are primarily intercellular and are not found in living cells of gall tissue on black raspberry. On the basis of limited observation this applies also to the tissues of crown galls induced on black raspberry.

STRUCTURE OF CYTOPLASM IN BLACK RASPBERRY CELL

The structure of cytoplasm has long been a subject of considerable controversy. Few of those who have interpreted this structure have made detailed studies of its contained bodies, namely, mitochondria, plastids, and fat globules, however, nor have they properly evaluated the effects of various fixing fluids upon these elements of the cytoplasm. In this way the reticular, filar, granular, and alveolar interpretations of this structure have arisen. GUILLIERMOND (**34, 42**) has emphasized the importance of a thorough understanding of these visible elements in evaluating cytoplasmic structure. He has shown that the cytoplasm of plant cells is a polyphasic substance composed of a ground substance, transparent and without visible structure in the living state, finely granular after fixation, and containing mito-

chondria, plastids, and fat globules. In the living cell these elements by their more general threadlike form, fat excepted, impart a structure to the cytoplasm that might be interpreted as fibrillar. An alveolar or foam pattern is generally induced by immersion of the cells in hypotonic solution, which incites the elements of the chondriome to swell so as to form a mass of soapbubble-like films. A coarse granular structure in the cytoplasm is generally induced by treating the cells with absolute alcohol, or the fluids of Carnoy, Bouin, and various mixtures containing acetic acid, which cause a partial disintegration of the chondriome.

Observations of the cytoplasm of living and degenerating cells of the black raspberry lead to reaffirmation of the views advanced by GUILLIERMOND relative to its structure. The reticular, filar, granular, and alveolar structures in the cytoplasm of these cells are products of the various states and arrangements of its contained bodies, namely, mitochondria, plastids, and fat. The cytoplasmic ground substance in freshly mounted cells has no visible structure. It has at most a faint, very finely granular appearance after fixation in mitochondrial fluids.

Summary

1. The bacteria which incite cane gall on fruiting canes of black raspberry are found primarily between the cell walls of infected tissue.

2. The bacteria ramify throughout all regions of cane gall tissue in the form of zoogleal strands which dissolve the middle lamellae of the cell walls.

3. Numerous visibly degenerating or necrotic protoplasts may be filled with bacterial thalli in certain areas of the gall tissue, and frequently the site of the protoplast within the walls is completely occupied by a swarm or solid mass of bacteria.

4. The bacteria also may occur in pockets or cavities which may be numerous and which result from lysis of masses of protoplasts and their cell walls.

5. The bacteria may be discharged from the intercellular spaces or cavities prior to disintegration of the gall.

6. Cells with which the bacteria are in contact undergo a more or less rapid cytolysis. In early stages of gall formation those cells at

a distance from the bacteria may divide repeatedly. Subsequently intercellular penetration may occur, and in turn the cells at a distance may be incited to extensive division. Eventually cell division ceases, extensive intercellular penetration by the bacteria continues, and the gall degenerates.

7. This degeneration is the result of two types of cellular disorganization and disintegration, cytolysis and autolysis.

8. During cytolysis the plastids are first reduced in size, are changed in form, and become indistinguishable from mitochondria. The mitochondria diminish in number and finally disappear from the cell. Coincident with lysis of the chondriome and reduction of cytoplasmic volume, the vacuolar volume of the cells increases and fat globules increase markedly in volume until they become the most conspicuous elements of the protoplast. The nucleus becomes granular, irregular in outline, and smaller until it finally disappears. At this stage fat and cell walls remain. Next the fat disappears and finally portions of the entire wall of the protoplast may be dissolved by the bacteria.

9. In autolysis the elements of the chondriome display a pronounced tendency to aggregate, and there is no appreciable increase in fat volume of the protoplast. Plastids and mitochondria generally fuse to form various reticular, solid condensation masses, or open, three dimensional networks of varying pattern. The elements of the chondriome may become vacuolate or they may fragment into granular débris. Ultimately all elements of the protoplast lose their identity in a granular or doughlike disintegrating mass of diminishing volume between collapsing cell walls, which in fixed material is stained intensely and which in fresh tissue is stained a faint red by Sudan III and brown by osmic acid.

10. The visible structure of the cytoplasm of healthy cells of the black raspberry or of those cells in which a pathic state is induced by the cane gall bacterium is the structure imparted to the cytoplasm by the various states and arrangements of its contained bodies, namely, mitochondria, plastids, and fat. In pathic cells this structure may be granular, alveolar, reticular, or it may appear as various three dimensional open network patterns. The visible structure of the cytoplasm of healthy cells such as those of root tips un-

affected by the cane gall bacterium is that of a polyphasic complex composed of a clear ground substance, without visible structure, in which the various spherical rodlike or threadlike mitochondria and spherical fat globules are dispersed and in which larger starch bearings plastids may also be dispersed or aggregated in various group patterns.

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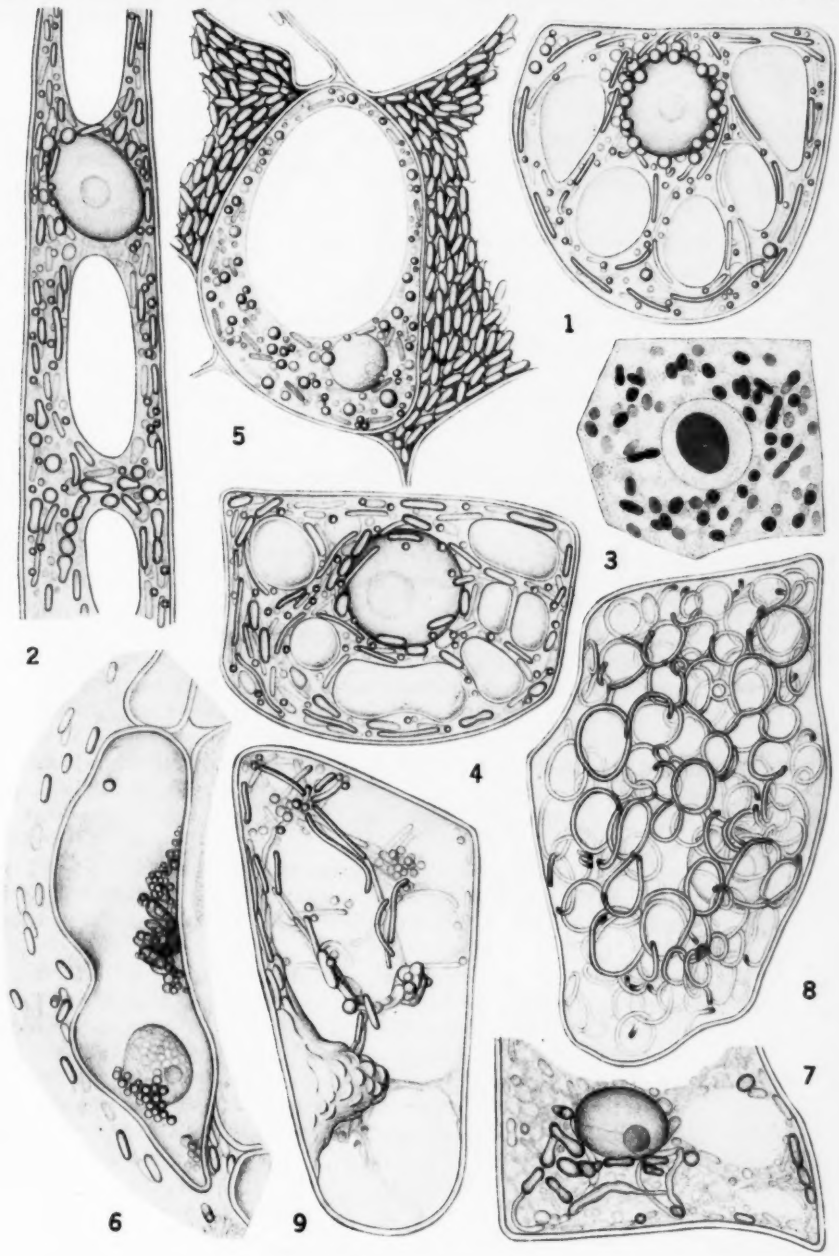


BANFIELD on CANE GALL

EXPLANATION OF PLATE I

All figures drawn at table level with Abbé camera lucida. Zeiss appochromatic 70×n.a. 1.25 water immersion objective with compensating 15 or 20× oculars used for figs. 1, 2, 4, 5, 6, 8, 9. Zeiss appochromatic 90× n.a. 1.3 oil immersion objective used for fig. 7; Spencer 100× fluorite objective used for fig. 3.

FIG. 1.—Typical cell from periblem of healthy root tip; the plastids are spherical, the mitochondria dispersed short threads. Fresh tissue section, unstained, 7.5% sucrose mount. ×1550 (approximately).

FIG. 2.—Typical cell from pleurome of healthy root tip as seen in unstained fresh section mounted in 5% sucrose solution; numerous plastids are of bacterial form and proportion. ×1550.

FIG. 3.—Cell from apical meristem of healthy root tip; plastids cannot be differentiated from mitochondria. NEMEC fixation, haematoxylin, balsam mount. ×2960.

FIG. 4.—Typical non-pathic gall tissue cell showing no visible difference between such cells and comparable cells of the root tip; elements of chondriome dispersed and very abundant; usually numerous plastids are of bacterial form and proportion; the small spherical fat globules are distinguishable by their higher refractivity. Fresh tissue section, unstained, 7.5% sucrose mount. ×2070.

FIG. 5.—Gall tissue cell recently encompassed by mass of bacteria; plastids have disappeared; mitochondria have diminished in number and the highly refractive fat globules have markedly increased in volume. Fresh section, unstained, 7.5% sucrose mount. ×2070.

FIG. 6.—Gall tissue cell which has been in contact with bacteria at edge of a bacterial cavity for a longer time; all elements of the chondriome have disappeared and fat globules are all that remain of the cytoplasm; the nucleus has a conspicuous granular appearance. Fresh section, unstained, 7.5% sucrose mount. ×2070.

FIG. 7.—Early stage of cellular disintegration from necrotic gall tissue. Some plastids have fused to form a reticulum of varying diameter. Erlicki fixation, haematoxylin, balsam mount, mitochondria not stained, not resolvable. ×2660.

FIG. 8.—Later stage of cellular disintegration from necrotic gall tissue. Dispersed elements of chondriome have fused to form a continuum or three dimensional netlike structure. Erlicki fixation, water mount. ×2070.

FIG. 9.—Plastids, mitochondria, and fat globules agglutinating into massive strand and solid condensation structures. Nucleus obscured by agglutinated mass of plastids over its surface. In autolyzing cells the identity of these elements frequently is lost in fusion structures. Fresh section unstained, 7.5% sucrose mount. ×2070.

DEVELOPMENTAL ANATOMY AND RELATIVE PERMEABILITY OF BARLEY SEED COATS

W. H. THARP

(WITH THIRTY-SEVEN FIGURES)

Introduction

Although much investigation has been centered upon determinations of the nature of the permeability phenomena of gramineous seed, most of it has been concerned only with the relative ability of various solutes to pass through the differentially permeable envelopes. Only two investigators have reported concerning the relative permeability of the grain itself. ORTON (17) has found varieties of *Zea mays* to differ in their relative permeability to several mercurial solutes in aqueous solution, and GUREWITSCH (13) has shown considerable variation in the degree of permeability among the individual kernels of an apparently uniform sample of one variety of wheat.

The investigations reported in this paper have been undertaken in an attempt to ascertain the extent of variation in the degree of permeability of the seed coat envelopes of common barley, special consideration being given the following questions: Do varieties of barley differ significantly in their relative degree of permeability? Does the environment during maturation influence the resultant permeability of the grain? Will prematurely harvested barley have an altered degree of relative permeability?

The seed of common barley, *Hordeum vulgare*, is known to be inclosed within a resistant, semipermeable envelope (3, 20, 7). However, a complete description of the origin, maturation, and ultimate condition of all the tissues contributing to the semipermeable envelope of the barley seed had not appeared in the literature at the time these investigations were begun.[1]

[1] The anatomical and physiological studies were started in 1929 at the University of Wisconsin and the investigations were continued while the writer held a National Research Council Fellowship at Cornell University, 1932–33. A preliminary report was presented before the physiology section of the Botanical Society of America at the annual meeting, Atlantic City, 1932 (23).

A thorough knowledge of the development of the caryopsis was deemed essential for the understanding of any effects that various changes of condition during growth might have upon its ultimate make-up.

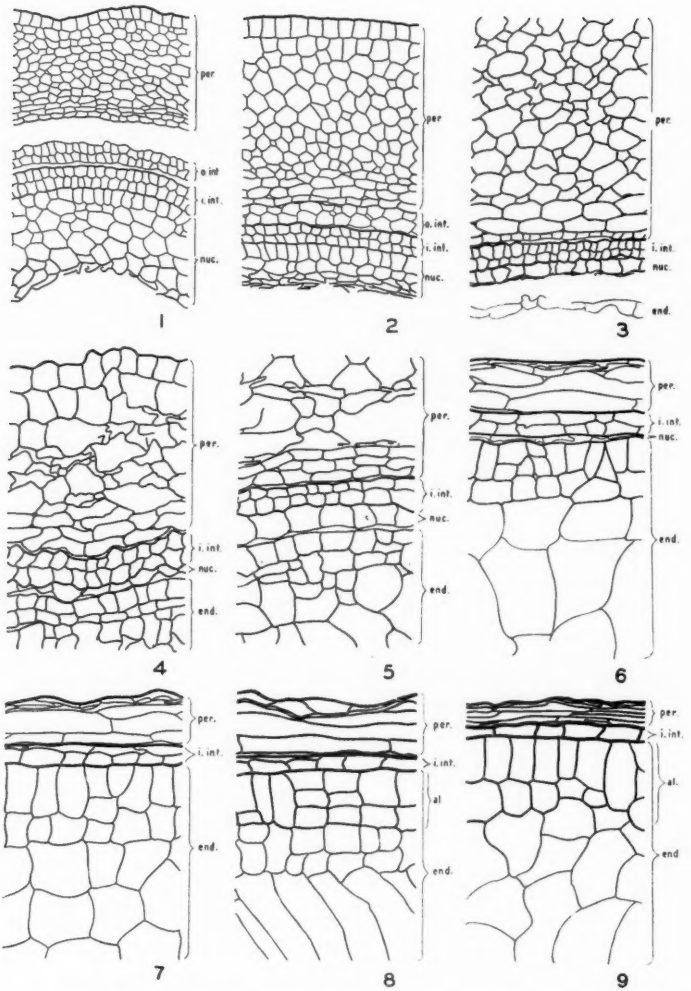
I. Morphological and anatomical studies

A. DETAILS AT TIME OF FLOWERING

MATERIALS AND METHODS

During the growing seasons of 1929 and 1930, heads of Oderbrucker barley (Wisc. Ped. 5–1) growing at the Hill Farms of Madison, Wisconsin, were marked at the period of pollination. Kernels were then gathered daily and prepared for sectioning. In addition to Oderbrucker, kernels of three other hulled varieties and of four hulless varieties were harvested at various stages of maturity. Transverse sections, 15 to 30 μ thick, of freshly harvested kernels were used for microchemical determinations. Heads and kernels to be imbedded in paraffin were killed and fixed in formal-acetic-alcohol in 1929 and in chrom-acetic medium in 1930. Materials were dehydrated by the ethyl alcohol-chloroform method in 1929 and by the butyl alcohol method in 1930. Silica was removed from the older heads and kernels by steeping for 36–60 hours in a solution of one part 50 per cent alcohol and one part commercial hydrofluoric acid. A combination of aqueous methylene blue, water-alcohol safranin, and orange G in clove oil was found satisfactory for the differential staining of sections of kernels gathered at the various stages of maturity. Sudan III in acidified 70 per cent alcohol and Sudan IV in lacto-phenol were employed to detect the presence of cutin and suberin. Microchemical analyses were made following the methods of ECKERSON (**9**) and HAAS and HILL (**15**). The accompanying illustrations, all prepared from microtome sections, consist of photomicrographs, drawings made with the aid of a camera lucida or a Promi projector, and enlargements of photomicrographs which were inked over and the residual photographic image then bleached away.

The single campylotropous ovule (**24, 16**) is attached near the distal end of the ovary and to the rachis side of the stylar canal by an elongate, reduced funiculus. The two integuments (fig. 1) are each two cell layers in thickness, except where the inner is bunched



FIGS. 1–9.—Transverse sections of Wisc. Ped. 5–1 barley kernels showing seed coats and peripheral tissues at mid-lateral surface: fig. 1, just before pollination; fig. 2, 2 days after pollination; fig. 3, 4 days after pollination; fig. 4, 7 days; fig. 5, 10 days; fig. 6, 13 days; fig. 7, 16 days; fig. 8, 20 days; fig. 9, mature kernel.

in the larger orifice of the outer integument at the micropyle (fig. 10). At this time there is no structural contact between the ovary, the two integuments, and the nucellar epidermis except at the chalaza. Sections of kernels at this age stained in acidified Sudan III show a thin red line of cutin on the inner and the outer epidermises of the ovary, a very faint trace of cutin on the inner and the outer epidermises of the outer integument, and a distinct cutin membrane on both the outer and the inner epidermises of the inner integument. The nucellar epidermis is without a definite cuticle at this stage. The main vascular supply to the ovary (usually seen as one large bundle but in many preparations distinctly of two separate and smaller bundles) passes up from the rachis, around the base of the ovary, and extends to the chalaza. The fruit is inclosed by the floral glumes and only partly fills the envelope formed by the imbricated lemma and palea.

B. DEVELOPMENT OF CARYOPSIS

ONE AND TWO DAYS FOLLOWING POLLINATION.—The developmental changes in the barley fruit are very rapid during the first two days following pollination. The kernel elongates rapidly and has attained its maximum length by the ninth day, while its maximum width is not attained until shortly before maturity. By the time the embryo consists of four cells, at the end of the second day (figs. 2, 14, 15), the micropyle has become closed and the entire nucellar epidermis is in contact with and adherent to the inner integument. The cutin membranes on the outer and the inner epidermises of the inner integument have thickened slightly and the cutin on the inner epidermis now seems to be common to both the integument and the nucellus. The growth of the inner integument has been accomplished mostly by cell division, little increase in cell size being noted. Although the outer integument has undergone much disintegration, the cells have been entirely absorbed only over the mid-abaxial surface. Increase in size of the pericarp has been mostly through enlargement of the cells, although in the region of the chalaza and the vascular bundle of the groove, cell division accounts for much of the growth and elongation. The inner epidermis of the pericarp is now well defined and the elongate cells adjacent to the epidermis mark the differentiation of the chlorophyll bearing tissue. The antipodal

cells and the free-nucleate endosperm form a thin layer in contact with the embryo and extend around the periphery of the enlarged nucellar cavity. The nucellus, except for the epidermis and one or two adjacent layers of cells, has been digested by the developing endosperm. In transverse sections of two-day-old kernels a group of glandular cells (the "sheaf cells" of COLLINS 7) can be discerned extending from the vascular bundle in the pericarp, through the chalazal region, and projecting fan-shaped into the nucellus.

THIRD TO SEVENTH DAYS.—By the fifth day of maturation the aleurone layer, composed of one to three rows of nearly rectangular cells, has been clearly differentiated. A primordial zone in the endosperm is now evident immediately interior to the aleurone. It is first active in the mid-posterior area but soon it initiates new cells from nearly the entire lateral periphery. By the seventh day of development there is a disorganized mass of endosperm and nucellar tissue in contact with the entire length of the chalaza. Another area of similar nature separates the endosperm and the still small (but slightly differentiated) embryo (fig. 18). These disorganized masses are composed partly of much hydrated cellulose and give a positive test for mucilage. The still persistent nucellar epidermis is in contact with the aleurone layer except where the latter is modified just interior to the chalaza and where it passes around the embryo as a single layer of cells. Cell division has now ceased in the outer row of cells of the inner integument, further enlargement of the integument being accomplished by the division of the cells of the inner row and elongation of the cells of both layers. The enlarged area at the micropyle has converged and the cutin membrane of the outer epidermis is now continuous over the micropyle. On the outer epidermis of the integument the cutin membrane has become somewhat thicker while the membrane on the inner epidermis has remained comparatively thin. In unstained transverse sections of five-day-old kernels (fig. 24), the walls of the cells in the chalazal region, and also some of the walls of the integument cells, are yellowish. This coloration is accompanied by change in wall composition, the cellulose becoming less easily hydrated and taking longer to dissolve in dilute, warm hydrochloric acid. The outer integument has entirely disintegrated and has been absorbed by the fifth day of development.

Within seven days the inner epidermis of the pericarp has also been mostly absorbed and the persistent portions crushed between the heavy walled chlorophyll tissue and the inner integument. There is now evidence of much crushing and breaking, with possibly some absorption, of the outer parenchyma of the pericarp (fig. 4).

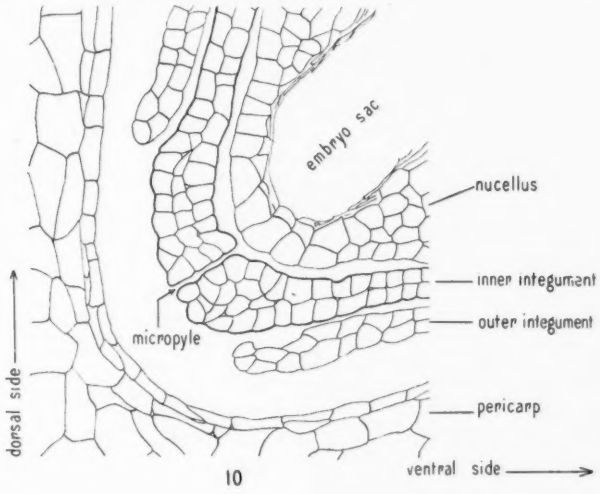
LATER WEEKS OF KERNEL MATURATION.—Continued centripetal development of endosperm, accompanied by progressive enlargement and differentiation of the embryo, is instrumental in causing the enlargement of the caryopsis during the second and third weeks of maturation. By the thirteenth day (fig. 6) following pollination the kernel has attained nearly its maximum length but only about one-half of its mature transverse circumference. Subsequent development and enlargement of the caryopsis are due entirely to increase in the endosperm, the cells of which become gradually filled with starch. The embryo, still very small although well differentiated by the twelfth day, has attained nearly its full size by the end of the third week. The cells of the aleurone layer attain their full size by the sixteenth (fig. 7) day, and any further maturation is accomplished by formation of the protein grains and by thickening of the soft, cellulosic cell walls. Suppression of the nucellus is practically complete by sixteen days, and during later stages it is hardly distinguishable from the thick outer wall of the aleurone layer. By thirteen days (fig. 6) the inner integument has become differentiated into an outer row of thin walled, elongate cells and an inner row of thicker walled, less elongate cells. Even as early as eight days a brownish deposit is evident in the cells of the transchalazal strand and in the inner row of cells of the integument. Unstained transverse sections of thirteen-day-old kernels (fig. 25) show most of the cells filled with the deposit. The inner cutin membrane of the integument has remained comparatively thin while the outer membrane has markedly increased in thickness. At sixteen days (fig. 23) the outer membrane appears about twice the thickness of the inner cutin membrane. Growth of the endosperm causes compression of the pericarp and subsequent flattening of the outer row of thin walled cells, there being little or no content in these cells by the end of the second week of development. The cells of the chalazal region are nearly all filled with the fat deposits by the twelfth day and each cell

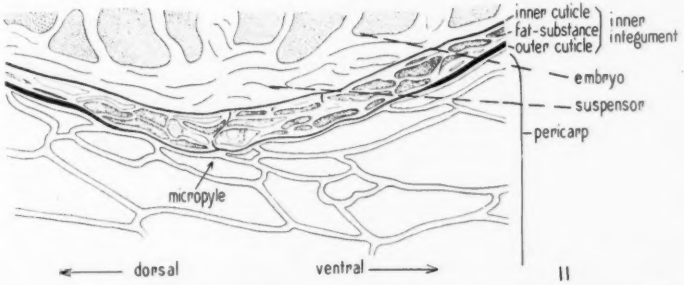
contains an inner suberin lamella, very thin but gradually thickening with maturity. The much elongated and very thick walled chlorophyll-containing cells are now the conspicuous portion of the pericarp.

MATURE KERNEL

INNER INTEGUMENT.—The mature caryopsis of barley (figs. 9, 20, 21) contains an embryo and endosperm completely inclosed by a resistant, cutinized, and semipermeable envelope (**4, 20, 7**). This membrane is composed of the inner integument, both inner and outer epidermises of which are cuticularized, and a suberized strand of chalazal cells connecting the flanks of the integument. The persistent inner integument is composed of two layers of cells. The outer epidermis of the inner integument, composed of a single layer of thin walled elongate cells which are nearly devoid of contents, has become crushed against the single layer of thick walled cells of the inner epidermis (fig. 9). In transverse section most of this integument thus appears to be composed of a single layer of cells with greatly thickened outer peripheral walls, since the lumena of the cells of the outer epidermis are discernible only in a few places where there has been less crushing. Most of the thicker walled cells of the inner row contain the remnants of their protoplasts, but for the most part the cells are filled with the fat deposits mentioned earlier. These deposits are found around the entire periphery of most kernels but in others they are absent from the cells over the anterior surface of the endosperm and sometimes over the embryo. Hull-less varieties, considered as a group, were found to have less of this substance deposited in the cells of the inner integument (figs. 27–29). It is also significant that the deposits were absent from the cells over the embryo, around the micropyle, and the abaxial surface of the endosperm more often in the hull-less than in the hulled varieties. The inner integument of the mature kernel is invested with a thin cutin membrane on its inner epidermis and a much thicker membrane on the outer surface (figs. 27–32). This membrane and the entire integument are not uniform in thickness. It is much the thickest where it folds over the apex of the kernel. It is very thick in the flanks of the furrow near the chalaza and is again thick near the grouped cells at the micropyle. It is slightly thinner over the lower anterior surface

than over the upper anterior and lateral surfaces, but it is distinctly thinner over the embryo. It is much the thinnest in the small area immediately opposite the micropyle (fig. 11).





FIGS. 10, 11.—Fig. 10, median, radial, longitudinal section through micropyle of barley kernel (Wisc. Ped. 5–1) just before pollination. Fig. 11, same of mature kernel of White Hull-less barley.

CHALAZA.—The cells of the chalaza are filled with fat deposits,[2] the walls are yellowish, and there is an inner lamella of suberin in each of these closely packed, thick walled cells.

[2] When the fat deposits are first formed they can be dissolved away with warm, alcoholic potassium hydroxide. As they increase in density and begin to fill the cells during

EMBRYO AND ENDOSPERM.—The embryo is oriented with its coleorhiza at the micropyle, pointing almost directly down parallel to the rachis, and makes contact with the micropyle by means of its group of empty suspensor cells. The scutellum of the embryo slants obliquely across the base of the kernel with its apex against the periphery at a point about one-third distant from the proximal end (fig. 20). The inner integument follows closely the curvature of that portion of the embryo which it directly covers. There is still a wide area of disorganized tissue between the scutellum and the starchy endosperm, clearly a result of digestion by the growing embryo, and a narrower line of similar nature extending the length of the chalaza and separating the "sheaf cells" from the aleurone layer.

ALEURONE.—The aleurone cells are thick walled and rectangular in outline, containing closely packed protein grains and rather large nuclei. The aleurone layer just interior to the "sheaf cells" is modified and its cells are somewhat disorganized. It passes around the embryo as a single layer of elongate cells, except at the micropyle where it ends at the margin of the compressed suspensor cells of the embryo.

NUCELLUS.—Remnants of the nucellus are discernible near the chalaza and over the top of the endosperm. Elsewhere this tissue has been absorbed or crushed until its remains appear to be part of the thickened peripheral walls of the outer row of aleurone cells. This layer of cells is now sealed to the cuticle of the inner epidermis of the inner integument.

PERICARP.—The thick walled cells of the pericarp are now sealed to the cuticle on the outer epidermis of the inner integument. The rest of the pericarp has been reduced, by absorption and crushing, to a few rows of collapsed parenchymatous cells and a much flattened outer epidermis with its cuticle. The vascular supply to the fruit

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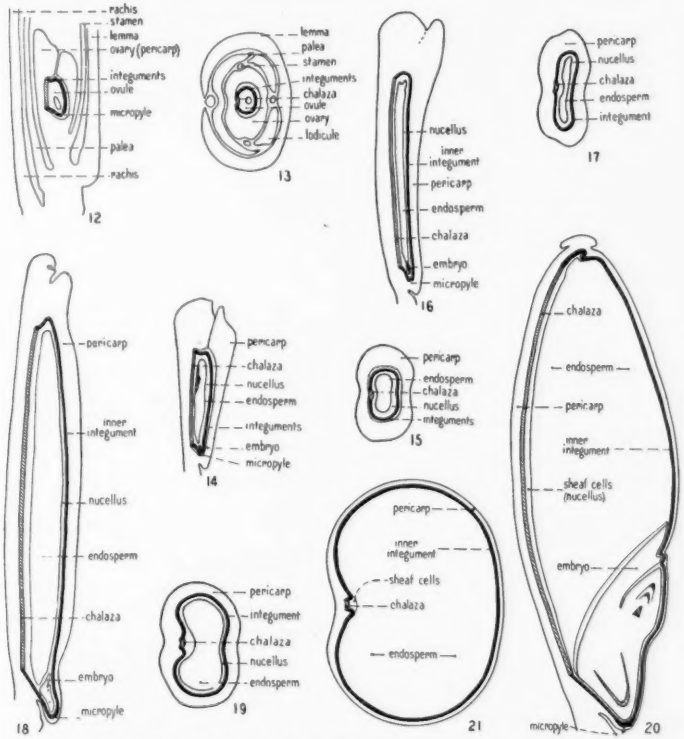
the later weeks of maturation, they become gradually more resistant to fat solvents. When the kernels are mature a prolonged heating in alcoholic potassium hydroxide will cause solution of much of this substance, but in the cells within and adjacent to the chalaza, and also in the cells around the micropyle, there often remain resistant outer lamellae of the original deposits. At no time during kernel development will this substance stain red in acidified Sudan III or Sudan IV, except for the very outer shell which may take the stain at its periphery. The exact composition of this substance has not been investigated during these studies.

extends the length of the groove, being surrounded on its posterior and lateral sides by crushed pericarp parenchyma and in contact on its anterior side with the glandular cells just exterior to the chalaza. The cuticle of the outer epidermis is adherent to the inner cuticles of the floral glumes where the latter are in contact with the caryopsis.

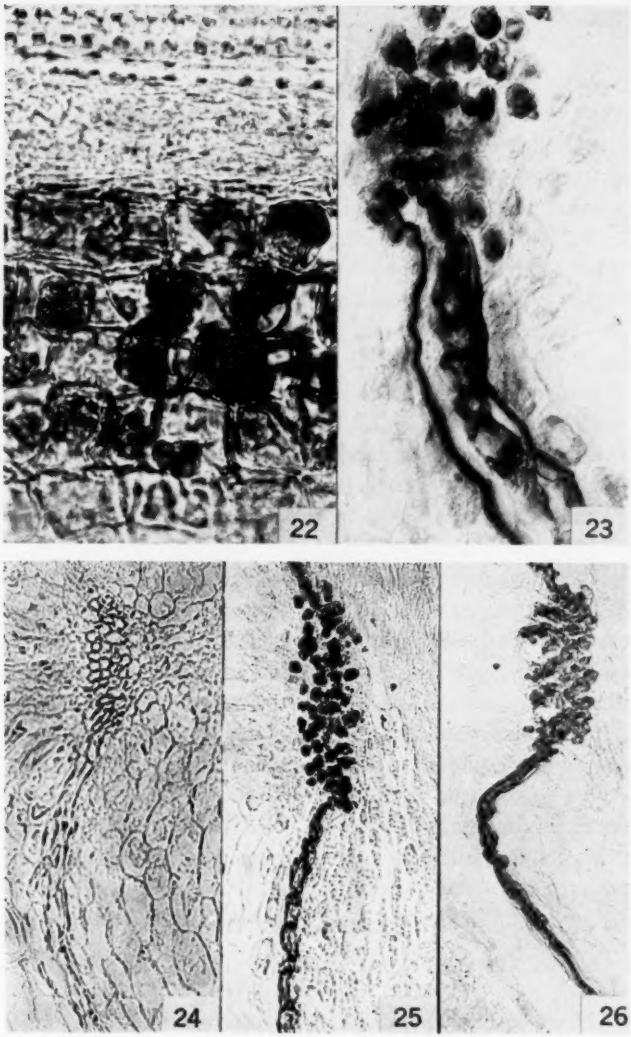


FIGS. 12–21.—Diagrams of longitudinal and transverse sections of Wisc. Ped. 5–1 barley kernels at five stages of development (palea and lemma have been omitted, except in figs. 12 and 13, of flowers fixed at time of pollination): figs. 14, 15, 2 days after pollination; figs. 16, 17, 4 days; figs. 18, 19, 7 days; figs. 20, 21, mature kernel.

PROBABLE RELATION OF SEMIPERMEABLE ENVELOPE TO VARIATIONS IN DEGREE OF PERMEABILITY

The results of these investigations are in agreement with those of COLLINS (**7**) and KRAUSS (**16**), in that the semipermeable envelope

of the mature caryopsis was found to consist of the inner integument, with outer epidermis heavily cuticularized and the inner epidermis lightly cuticularized, and the resistant suberized cells across the length of the chalaza. It is also agreed that the thickness of the outer cuticle of the integument is not uniform over the entire seed, it being relatively thin in the region over the embryo. These previous investigators have found also that the two membranes were originally developed by the integument before the time of flowering. COLLINS has suggested that the tract of chalazal tissue where it meets the dorsal rim of the scutellum might be a possible point of easy permeability. The present investigations and those of KRAUSS have discovered no structural weakness at this point indicative of easy permeability, but rather that the thin membrane across the micropyle (COLLINS' second point of probable easy permeation), and to some extent the relatively thin covering over the embryo, are structurally the most logical points of probable easy permeation. KRAUSS has pointed out that in naked barleys the cells of the integument at the micropyle do not usually contain the fat deposits found elsewhere in this tissue. He has also suggested that in some varieties the outer cuticle and at times the inner cuticle may be entirely absent from the micropylar area. In the four hull-less varieties (C. I. nos. 4360, 4344–2, 4339–1, and White Hull-less) used in these investigations the micropylar area is usually crossed by at least a very thin membrane on the outside and on the inside of the integument at this point. It is true that the cutin deposit of the integument is relatively more apparent in immature kernels but seldom is it absent from this area of the mature kernels investigated. In some hull-less varieties there are found more kernels with but little of the fat substance in this group of integument cells, but in any variety studied the deposits are more often absent from the cells over the embryo and the anterior surface than from the region across the micropyle. KRAUSS has reported that in many kernels of naked barley the cells of the integument at the micropyle could be dissolved away with strong acids which have little effect on the cutin membranes which he believed to be absent in these cases. The writer steeped 20 mature kernels of each of the four hull-less varieties investigated in 60 per cent hydrochloric acid for 24 hours at room temperature. From one



FIGS. 22–26.—Fig. 22, longitudinal section through chalazal region of mature kernel, stained in acidified Sudan III. Note distinct suberin lamella of chalazal cells and large deposits of fatty substance. Vascular bundle of pericarp seen at top of photograph. ×1250. Fig. 23, transverse section of kernel fixed 16 days following pollination, stained in acidified Sudan III. The two cutin membranes of integument extend well into chalaza. Pericarp is toward left of photograph. ×580. Figs. 24–26, unstained, transverse sections of barley kernels showing natural staining of cell walls and fat deposits within cells of inner integument and chalaza: fig. 24, fixed at 5 days; fig. 25, at 13 days; fig. 26, at maturity. Pericarp toward right of photographs. ×270.

to three of the kernels of each variety had the embryo entirely dissolved by the end of this time. The remaining 17–19 kernels of each lot were swollen and distended over the embryo end, and subsequent examination of the embryo showed it to be unaltered by any solvent action of the acid. It must then be concluded that about 90 per cent of the kernels of any variety investigated[3] contain sufficient cutin deposited over the micropyle to inhibit the entrance of 60 per cent hydrochloric acid for 24 hours and thus demonstrate the possession of semipermeability dependent here upon the cutin membranes.

In each of the eight varieties of barley investigated, it has been found that from the time of flowering to maturity there was a gradual and progressive thickening of the outer cutin membrane of the inner integument. It might then be expected that prematurely harvested kernels would contain a thinner deposit of cutin on the integument than fully ripened kernels, and subsequently be more readily permeable. It was found also that there was a relative difference in the thickness of these membranes among different varieties of barley, again suggesting a variation in degree of relative permeability. Another variable which might be responsible for resultant differences in permeation rates is the amount of the fat deposits within the cells of different areas of the integument. While the comparative differences of varieties relative to each of these factors suggest a possible correlation of permeation with structure, it might again be possible that there exist within the cutin membranes some differences in chemical and physical composition which are the controlling factors concerning the subsequent relative permeability of the seed envelopes.

II. Permeability studies

A. RELATIVE RATES OF ABSORPTION FROM AQUEOUS SOLUTIONS BY BARLEY KERNELS

MATERIALS AND METHODS

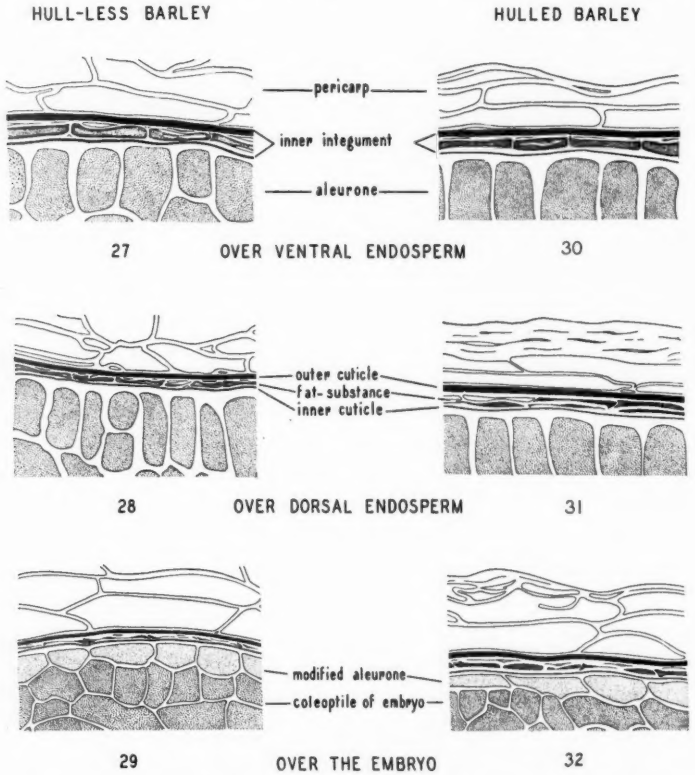
The same eight varieties of barley used for the morphological studies were employed in the permeability investigations. In the summer of 1930, and again in 1931, one lot of each variety was grown under prevailing cultural conditions at the Hill Farms, Madison,

[3] These varieties of hull-less barley are not the same as those used by KRAUSS (16).

Wisconsin. Another lot of each variety was grown under a field moist-cage where high humidity, high soil moisture content, and low light intensity were maintained throughout the period from flowering to complete maturity. The samples grown under field conditions are here termed as from a "dry" environment and those matured under the moist-cage as from a "wet" environment, these terms being only relative. Four harvestings were made from each of the varieties grown in the two environments as follows: at 14, 21, and 28 days following flowering, and at maturity. A portion of each prematurely harvested sample was preserved in 70 per cent ethyl alcohol and the remainder of the samples were allowed to air-dry in the laboratory.

The increase in weight of kernels steeped in various solutions has been used by BROWN (**3, 4**) and many other investigators as a measurement of the rate of permeation of solvent and solute. This method has been adopted, with modifications, to determine the differences in permeability among different varieties of barley and also those differences induced by the environment during maturation. Three different steeping solutions have been employed: (1) distilled water which permeates rapidly, (2) an aqueous solution of iodine-potassium iodide which permeates but fairly rapidly, and (3) various concentrations of sodium chloride which permeate with exceeding difficulty. Duplicate 100-kernel samples of each lot were steeped at 25° C. in 250 cc. Pyrex beakers containing 150 cc. of the solution. Those samples steeped in water and in iodine-potassium iodide were removed at regular intervals of 15, 30, 45, and 60 minutes and then at the end of 2, 3, 6, 9, 12, 24, and 32 hours. The samples steeped in distilled water were drained and then dried by rolling between towels for one minute. They were then centrifuged at high speed for one minute in wire baskets over absorbent cotton (fig. 33) and weighed within the first minute following removal from the centrifuge. Since it is absorption by the seed, exclusive of that absorbed by the pericarp, that it is desired to calibrate, this method aids in rapidly freeing the pericarp of moisture while the semipermeable membranes inhibit any appreciable loss from the seed. The samples steeped in a solution of 1 per cent iodine in 10 per cent potassium iodide were drained and immediately washed in one change of water. These

washed kernels were placed in a 10 per cent solution of sodium thio-sulphate for three minutes, the time necessary to clear most of the iodine from the tissues of the pericarp. The cleared kernels were washed in four changes of tap water and then dried and weighed



FIGS. 27–32.—Transverse sections of two varieties of barley showing structure of mature seed coat membranes over ventral (posterior) and dorsal (anterior) endosperm, and over embryo: figs. 27–29, White Hull-less barley; figs. 30–32, Wisc. Ped. 5–1 barley.

with the same procedure. The following methods were employed to determine the effect of concentration of sodium chloride upon the relative absorption by different lots of barley. Duplicate 100-kernel samples of each lot were placed in each of nine differing concentra-

tions of sodium chloride and all samples were steeped for 24 hours at 25° C. At the end of this time the samples were drained, washed in running water for three minutes to free the salt from the tissues of the pericarp, then dried and weighed following the same technique used for the samples steeped in water. It was found that the kernels taken from the concentrated salt solutions would increase in weight during the three minute washing. To eliminate the discrepancy in-

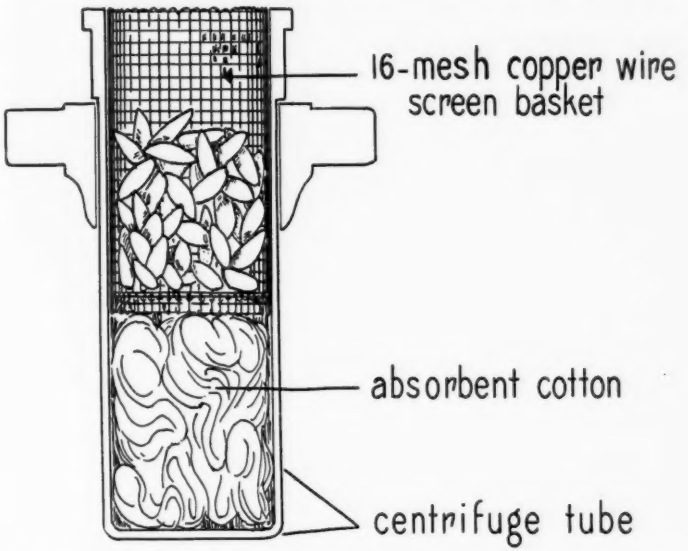


FIG. 33.—Centrifuge tube-holder adapted for rapid removal of adhered moisture from steeped barley kernels.

troduced by this procedure, the average amount which air-dry seed, of the same lot, would absorb during this after-treatment was subtracted from the total amount absorbed. This correction was applied to the result from each sample, even the ones steeped in zero sodium chloride (water). Table I gives the data obtained in one of these experiments using samples of Spartan barley, from both the wet and the dry environments, steeped in distilled water. The results of all the steeping experiments are represented graphically by figure 34.

It is obvious, considering the results shown in table I and figure 34,

that a relatively wet environment during maturation induces a greater degree of permeability of the seed coats and a greater absorptive capacity of the seed. At any one time after the first 15 minutes of steeping there has been more water absorbed by the wet than by the dry sample of any of the varieties tested. Another interesting relation is shown when the grain is allowed to absorb water until a constant weight has been attained. When the samples have thus

TABLE I

ABSORPTION OF WATER BY TWO DIFFERENTLY MATURED LOTS OF SPARTAN BARLEY (25° C.)

Time of steeping	Dry maturation			Wet maturation		
	Weight of 100 kernels (gm.)			Weight of 100 kernels (gm.)		
	Before steeping	After steeping	Percentage gain	Before steeping	After steeping	Percentage gain
15 sec.....	4.9931	5.3483	7.11	4.4109	4.8142	9.14
30 "	5.0000	5.4626	9.25	4.4390	4.9581	11.69
45 "	5.1026	5.6957	11.62	4.4413	5.0221	13.07
60 "	4.9757	5.6087	12.72	4.2810	4.9364	15.30
2 min.....	4.8244	5.6235	16.56	4.4248	5.2628	18.93
3 "	5.0037	5.8975	17.86	4.4863	5.4539	21.56
6 "	5.0007	6.1932	23.84	4.3766	5.7271	30.85
9 "	5.1516	6.5964	28.04	4.4370	5.9560	34.23
24 "	5.0158	7.2798	45.13	4.2370	6.5673	53.69
33 "	5.0521	7.6864	52.14	4.4122	7.0600	60.01

reached an equilibrium with the solution, the wet samples of each variety have absorbed a greater amount of water than the comparable dry samples, even though the average weight of the wet samples is less than that of the dry samples. A comparison of the curves of absorption by Wisc. Ped. 38 barley shows that in a given variety the more rapid rate of permeability and the greater absorptive capacity of the wet samples remain relatively constant, whether the kernels have been steeped in pure water (permeating easily), in iodine-potassium iodide (permeating less easily), or in sodium chloride (scarcely permeating). Each variety tested showed a different relative degree of permeability proportionally maintained in the three types of solutions. The differently matured samples of the varieties

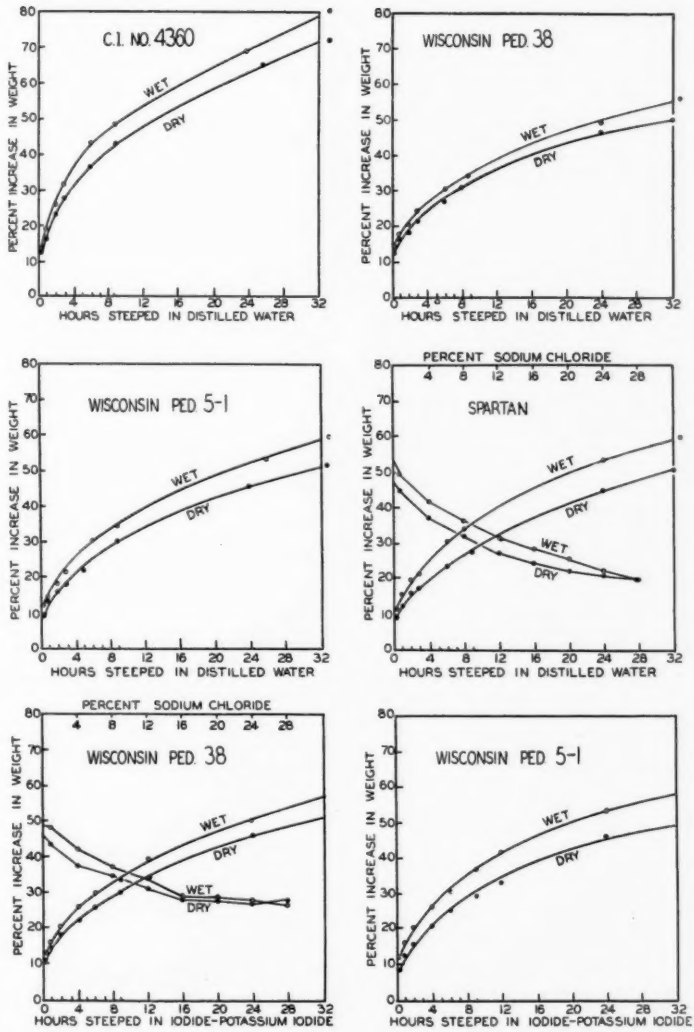


FIG. 34.—Relative rates of absorption from aqueous solutions by barley kernels (temperature 25° ±1° C.). Each point on curves represents average absorption by two 100-kernel samples. Samples of wet kernels were matured under field moist-cage; dry samples were matured under prevailing cultural conditions.

steeped in the sodium chloride solutions exhibited divergent degrees of absorption in the dilute solutions but failed to differ materially in this respect when steeped in the more concentrated solutions.

B. PERMEABILITY OF BARLEY SEED COAT MEMBRANES

GOLA (**11, 12**), SHULL (**21**), DENNY (**8**), ORTON (**17**), and others have employed seed coat membranes as septa of variously constructed osmometers. This procedure furnishes an accurate method of determining the permeability of the seed coat without involving other complex forces operative during absorption. These methods of diffusion are rather slow, and because considerable variation is encountered in any one carefully selected lot of grain, it was desired to find a method whereby large numbers of membranes could be tested for any one determination.

OSTERHOUT (**18**) has measured the permeability of living cells by placing together many discs of tissue and then testing their resistance to electrical conductivity. STILES and JØRGENSEN (**22**) have criticized this method on the assumption that an electric current would induce marked changes in the protoplasmic membranes of the living cells. This objection is of course not applicable to the nonliving semipermeable membranes of the barley kernels. GUREWITSCH (**13**) has adopted the OSTERHOUT principle using seed coat membranes of wheat as septa in an osmometer with the electrodes completely separated by the membrane. He tested one variety of wheat kernels in many solvents and his results seem significantly quantitative when great numbers of membranes from any one sample were tested for one average determination. This last method has been adopted, in somewhat modified form, for these investigations.

C. RESISTANCE OF SEED COAT MEMBRANES TO CONDUCTIVITY IN SOLUTIONS OF ELECTROLYTES

MATERIALS AND METHODS

About 150 membranes were prepared from each of the variously harvested samples of the eight varieties of barley used in these experiments. Membranes were also prepared from the kernels, which were killed and preserved in alcohol immediately after premature

harvesting. The carefully selected kernels were soaked in water at 32° C. for twelve hours, after which time the hulls could be removed and the endosperm was sufficiently soft to be cut easily. The tip and base of each kernel were cut off transversely and the hardened tissue of the groove removed by a longitudinal cut along each flank. These cut kernels were placed in a 10 per cent solution of taka-diastase and allowed to digest at 38° C. for 60 hours. The softened starchy endosperm was then easily removed and the membranes were thoroughly washed in running water. The finished membranes were stored in 30 per cent ethyl alcohol until tested. Each prepared membrane consists of the following tissues: (1) the intact aleurone with fragments of the endosperm cells; (2) the remnants of the nucellus; (3) the inner

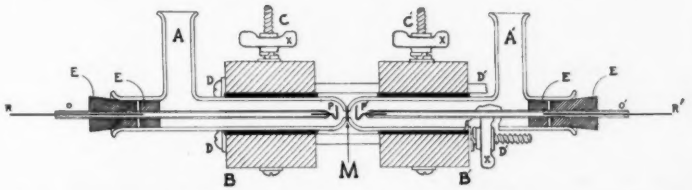


FIG. 35.—Special cell for testing resistance of barley seed coat membranes to electrical conductivity in solutions of electrolytes: *M*, test membrane placed between ground edges of two tubes (*A*,*A'*) and tightened in place by four set screws (*D*,*D'*). The tubes of Pyrex glass are filled with test solution through openings at *A* and *A'*. The two platinum electrodes (*ROP*, *R'O'P'*) are held in place by sealed-in stoppers (*E*). Tubes held firmly in the blocks by hinges on one end and by set screws (*C*,*C'*) on the other end. Tubes may be separated for insertion and withdrawal of membranes by loosening set screws (*D*,*D'*). Current supplied at electrode leads (*R*,*R'*). Conductivity surface of electrodes (*P*,*P'*) is about 18 sq. mm. Electrodes operate at a distance of 23 mm.

integument with its thin inner and thick outer cutin membranes; and (4) the pericarp with the epidermis often torn away during preparation. The aleurone and pericarp were left on the finished membranes because their added thickness facilitated the sealing of the membranes between the two ground edges of the osmometer tubes. Actual tests have demonstrated that the removal of either or both of these layers causes only a negligible change in their resistance to conductivity. The apparatus designed and used for testing the resistance of these seed coat membranes to conductivity is shown and described in figure 35.

TESTING RESISTANCE

Each lot of membranes to be tested was removed from the alcohol, thoroughly washed, and then placed in distilled water. In conducting an individual test a membrane was first steeped in the test solution for three minutes in order to obtain complete infiltration. After this pre-soaking the membrane was securely clamped between the ground edges of the tubes, the tubes wiped dry at their junction, and each tube filled with solution so as to cover the electrodes completely. The wheatstone bridge was then connected to the electrodes and the resistance determined by the Kohlrausch method (10). While one reading was being taken the next membrane was placed to soak, and so on. Special care was exercised when inserting a membrane so as to avoid leakage during the test, because even a minute leak at this juncture would result in a capillary film of liquid around the membrane edge, thus lowering the resistance reading to a noticeable degree. Considerable variation within any one lot of barley membranes, or even at different locations on the same membrane, necessitated twenty or more separate determinations for every lot of membranes tested. These initial readings were later checked with additional readings using a second group of membranes prepared from the same lot of barley. If any discrepancies were noted between these two sets of data, additional readings were taken until an accurate average could be established. Trichloracetic acid has been chosen as a test solution because it is known to be an electrolyte which permeates easily (4). Other electrolytes have been used to establish the fact that the results are not peculiar to the one solution.

The data in table II and the resistance graphs in figure 36 indicate that for each variety of barley tested there is a definite, average degree of permeability; that Spartan is the least permeable and C. I. no. 4360 the most permeable, with the other varieties exhibiting various degrees between the two extremes; that the effect of increased moisture, high humidity, and low light intensity during maturation is a greater degree of permeability in all the varieties except C. I. no. 4339–1.[4] The results obtained with three of the

[4] Similar tests of the differentially matured samples of this variety, using materials grown the preceding year (1930), gave a difference in the same relative proportion

varieties, grown under the two differing environments and harvested at the various periods during growth, are presented graphically in figure 37. The data are not complete for the entire series, nor for any one variety, because in many cases the membranes stored in alcohol were dried down and could not be tested comparably with the rest of the stored samples.

TABLE II

RELATIVE RESISTANCE OF EIGHT VARIETIES OF BARLEY, MATURED UNDER TWO DIFFERENT ENVIRONMENTS, TO ELECTRICAL CONDUCTIVITY IN NORMAL TRICHLORACETIC ACID (25° ± 1° C.)

SAMPLE	NUMBER OF MEMBRANES TESTED	READINGS IN OHMS RESISTANCE		
		HIGHEST	LOWEST	AVERAGE
Spartan				
Dry	20	2300	1200	1782
Wet	25	1900	930	1373
Wisc. Ped. 5–1				
Dry	32	1980	1100	1663
Wet	27	2000	1000	1399
Trebi				
Dry	33	1900	1000	1401
Wet	30	1900	900	1246
Wisc. Ped. 38				
Dry	36	2000	900	1341
Wet	41	1900	800	1199
C.I. no. 4344–2				
Dry	31	1700	1100	1329
Wet	33	1400	1000	1172
C.I. no. 4339–1				
Dry	33	900	620	762
Wet	36	990	450	764
W. H.				
Dry	30	800	510	660
Wet	24	720	520	631
C.I. no. 4360				
Dry	30	580	300	408
Wet	32	420	280	336

These data show that there is an increase in resistance of the seed coats correlated with the increased periods of maturation. That there is an early effect of the environment upon the subsequent permeability is shown by the differences in resistance of the samples of

shown by other varieties grown in 1930 and in 1931. The nearly exact average resistance of the membranes of the two lots may be due to the fact that they were inadvertently prepared from the same lot of barley.

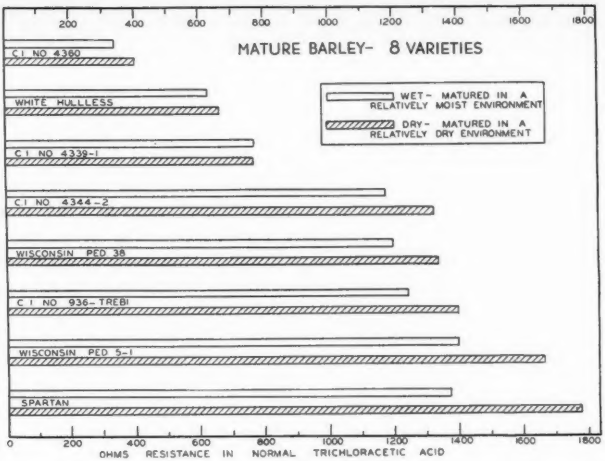

MATURE BARLEY- 8 VARIETIES
WET- MATURED IN A RELATIVELY MOIST ENVIRONMENT
DRY- MATURED IN A RELATIVELY DRY ENVIRONMENT
C.I. NO 4360
WHITE HULLESS
C.I. NO 4339-1
C.I. NO 4344-2
WISCONSIN PED 38
C.I. NO 936-TREBI
WISCONSIN PED 5-1
SPARTAN
200 400 600 800 1000 1200 1400 1600 1800
0 200 400 600 800 1000 1200 1400 1600 1800
OHMS RESISTANCE IN NORMAL TRICHLORACETIC ACID

FIG. 36

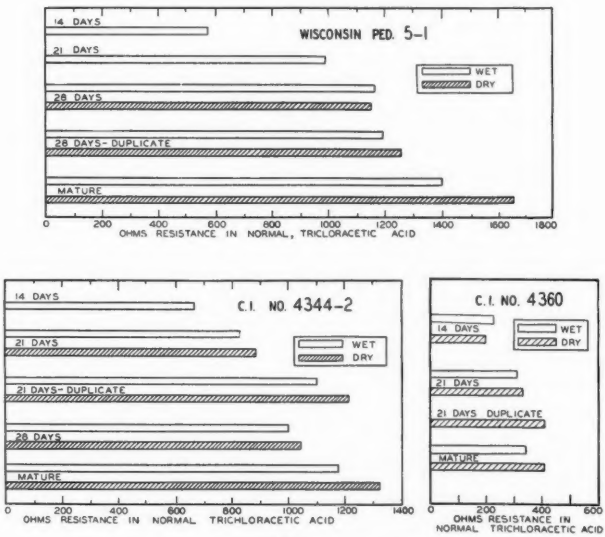


FIG. 37

FIGS. 36, 37.—Fig. 36 (above), relative resistance to conductivity of differently matured samples of eight varieties of barley. Resistance tested in normal trichloracetic acid at 25° ±1° C. Each column represents average resistance of 25 or more individual membranes. Fig. 37 (below), relative resistance to conductivity of differently matured samples of three barley varieties, harvested at 14, 21, 28 days following pollination, and at maturity. All samples preserved in 70% alcohol when gathered, except 'Duplicate' samples which were first allowed to become fully air-dried. All samples tested in normal trichloracetic acid at 25° ±1° C.

C.I. nos. 4360 and 4344–2 which were harvested at 21 days following flowering. Another striking result is evident in the relative resistance of the samples which were prematurely harvested and then allowed to dry (duplicate). The resultant resistance to permeation is higher than that of the comparable samples which were preserved in alcohol when harvested and is nearly as high as that of the fully matured samples. The results of an early harvest are not the same for each of the varieties tested although the general trend is similar. It should be noted that the samples of Wisc. Ped. 5–1 and of C.I. no. 4344–2 have a similar relative resistance at fourteen days, and then at maturity their permeability values are divergent. The samples of C.I. no. 4360 at any chosen stage of development were found to be much more permeable than the comparable samples of the other two varieties. It is evident that there is a wide divergence in permeability among different barley varieties at maturity, and that the rate at which resistance of the seed coats is increased during maturation is not the same for all varieties. This might be due, in some measure, to the fact that the high resistance of the seed coat membranes of some varieties is a possible development of the later part of the maturation period.

RESISTANCE OF BARLEY SEED COAT MEMBRANES TO CONDUCTIVITY IN SOLUTIONS OF INORGANIC ELECTROLYTES

The relative differences in seed coat permeability, which were obtained with trichloracetic acid as the test solution, were encountered when tested in solutions of inorganic electrolytes. The data in table III give a comparison of the results obtained with two lots of membranes tested in sodium chloride and in trichloracetic acid. A comparison of the relative resistance of one lot of membranes to conductivity in trichloracetic acid and in various solutions of inorganic electrolytes is shown by the data presented in table IV.

The data in table IV show that significant differences in relative permeability of seed coat membranes can be demonstrated by the use of solutions of solutes which permeate with difficulty. The differences are not exactly proportional in relation to those obtained using trichloracetic acid, it being obvious that non-permeating solutions such as sodium chloride do not offer an accurate means of determin-

ing slight differences by the conductivity method of testing. The inorganic acids tested permeate more slowly than trichloracetic acid, even though the concentrations were adjusted to give the same

TABLE III

RELATIVE RESISTANCE TO CONDUCTIVITY OF TWO SAMPLES OF BARLEY SEED COAT MEMBRANES TESTED IN TRICHLORACETIC ACID AND IN SODIUM CHLORIDE (25° C.)

Sample of membranes	Tested in 2.n sodium chloride			Tested in normal trichloracetic acid		
	No. of readings	Readings in ohms resistance		No. of readings	Readings in ohms resistance	
		Without membrane	Aver. of membranes		Without membrane	Aver. of membranes
C. I. no. 4360 wet	20	37	6,770	20	29.2	389
Trebi dry	20	37	9,850	20	29.2	1330

TABLE IV

RELATIVE RESISTANCE TO CONDUCTIVITY OF SEED COAT MEMBRANES FROM WISC. PED. 5–1 BARLEY IN SOLUTIONS OF INORGANIC ACIDS, BASES, AND SALTS (25° C.)

Solution	Concentration	No. of readings	Readings in ohms resistance	
			Without membrane	Aver. of membranes
Trichloracetic acid	1.n	24	31.2	1,368
Nitric acid	0.275 n	24	31.2	2,770
Hydrochloric acid	0.25 n	24	31.2	3,600
Potassium hydroxide	0.5 n	12	31.2	3,150
KOH (membranes steeped in solution 5 min.)	0.5 n	12	31.2	776
Potassium iodide	10%	24	78	9,840
KI plus 1% I_2	10%–1%	24	147	1,890

initial conductivity reading of 31.2 ohms. Potassium hydroxide, also with the same initial reading, encounters a similar high resistance in the membranes until it has apparently had some solvent action upon the cutin membranes (evidenced by the lower resistance after pre-

soaking). Since iodine-potassium iodide has been found to permeate readily in absorption experiments, it is rather to be expected that it is found to permeate more rapidly in these tests than the potassium iodide having a much lower initial conductivity (table IV). Just why the addition of the metallic iodine should increase the permeability to the resultant solution is not fully understood, but it seems possible that it may be due partially to the complex nature of the double salt thus formed.

The data in table IV show a probable solvent action of potassium hydroxide when the membranes were immersed in the solution for a

TABLE V

EFFECT OF PRE-TREATMENT WITH LIPOID SOLVENTS UPON THE SUBSEQUENT RESISTANCE OF SEED COAT MEMBRANES FROM WISC. PED. 5–1 BARLEY TESTED IN NORMAL TRICHLORACETIC ACID (25° ±1° C.)

(CHECK RESISTANCE, WITHOUT MEMBRANE, 31.2 OHMS)

TREATMENT	NO OF READINGS	READINGS IN OHMS RESISTANCE		
		LOW	HIGH	AVERAGE
Untreated	24	900	1,900	1,368
Refluxed in ether 2 hours	24	800	1,800	1,230
Refluxed in alcohol KOH for 2 hours	24	120	900	388

short period. These results led to testing the effect of pre-treatment in several fat solvents upon the subsequent membrane permeability. One lot of membranes from Wisc. Ped. 5–1 dry barley was refluxed for two hours in a solution of 2 per cent potassium hydroxide in 60 per cent alcohol; the membranes were washed thoroughly before testing. Another lot of the same membranes was refluxed for two hours in ethyl ether, hydrated through dilutions of alcohol, and then washed. These two lots and an untreated lot of the original sample were tested for their relative resistance in trichloracetic acid. The results are assembled in table V.

It is seen that the treatment in ether little alters the resistance of such membranes. The treatment in potassium hydroxide, however, causes a marked lowering of the resultant resistance. The solvent action of the alkali has evidently removed or altered the major por-

tion of the substances responsible for the relatively high resistance of these membranes. The fact that little of the substances making for the resistance were removed or altered by the ether treatment lends strength to the assumption that there is little true fat in the make-up of these semipermeable cutin membranes, or if so that it is not responsible for relative impermeability.

D. RELATIVE PERMEABILITY OF VARIOUS AREAS OF SEED ENVELOPE OF MAIZE

METHODS

The grain of *Zea mays* has been reported as possessing a semipermeable envelope in many respects similar to that of barley and wheat (**21, 1, 17**). The membranes taken from barley kernels being

TABLE VI

RESISTANCE OF SEED COAT MEMBRANES FROM DIFFERENT AREAS OF KERNELS OF TWO VARIETIES OF MAIZE TESTED IN NORMAL TRICHLORACETIC ACID AT 25° C.

(CHECK READING 40 OHMS)

VARIETY	PORTION TESTED	NO. OF READINGS	READINGS IN OHMS RESISTANCE		
			HIGH	LOW	AVERAGE
Yellow Dent	Top of kernel	25	660	410	515
Yellow Dent	Posterior	25	790	470	612
Yellow Dent	Portion over embryo	26	380	230	303
Blue Aleurone Cornell no. 633–13	Anterior	24	460	280	368
Blue Aleurone Cornell no. 633–13	Portion over embryo	28	220	247	176

rather small, it was decided to use the membranes from maize to determine the relative resistance of the various areas of the seed covering.

Kernels of Yellow Dent and Cornell no. 633–13 (Blue Aleurone), both in inbred lines, harvested just before complete hardening of the starch had resulted, were steeped for twelve hours in water at 32° C. The kernels were then cut into three portions: a top (apical) portion was cut off transversely and the remainder was split into a posterior

portion and an anterior portion containing the covering over the embryo. Membranes were prepared from these three portions, using the technique adopted for the preparation of the barley membranes, and their relative resistance to electrical conductivity was tested in normal trichloracetic acid. The data are assembled in table VI.

ORTON (17) has found that the rate of permeability of corn seed coat membranes to mercurial compounds in aqueous solutions varies with the variety of corn tested, and "apparently that portion of the seed coat covering the embryo side of the seed is more slowly permeable than that part covering the endosperm side." The results obtained in these experiments are in agreement with ORTON in part only. That there is a difference in degree of permeation among varieties is evident, but the data in table VII offer rather substantial evidence that the portion of the seed coat envelope covering the embryo is much more easily permeable (at least to this solution) than the covering of the endosperm, either over the apex or over the posterior surface (rachis side).

Discussion

A gradient of permeability to iodine in wheat grain coats was postulated by BRAUN (2) in disagreement with the conclusions arrived at by HARRINGTON and CROCKER (14). The latter, working with Johnson grass, had stated, "The solute enters only slightly through the surface of the grain but largely through a hilum or micropyle and spreads laterally and in a distal direction under the seed-coat." SCHROEDER (20) previously had suggested that absorption in wheat was exclusively at the embryo with the subsequent spread through the grain within the semipermeable envelope. COLLINS (7) also believed that only a small part of the water absorbed by the grain of barley entered by the general surface, special points of entry being in the germinal region. BEESKOW (1) and SHULL (21) found with maize that the diffusion of permeable solutes (iodine) through the membranes covering the endosperm is more rapid than any spread within. BROWN (5) found that the absorption of water by the seed of *Lolium perenne* is first in the germinal region, at the micropyle, and the slight upward spread within the coats causes a progressive swelling and stretching of the coats with a resultant in-

creased permeability at progressively higher levels. He later (**6**) related the same theories to the phenomena of absorption by the grain of wheat. Previous anatomical and morphological studies have shown that in each of the thus investigated Gramineae the seed is inclosed by a semipermeable cutinized (or suberized) envelope (**7, 20, 21, 19, 16**).

The present investigations have demonstrated that in barley grain the rate of absorption by the entire kernels can be correlated with the relative rates of permeability of the seed coats covering the anterior portion of the endosperm. These comparative relations have been demonstrated in solutions which permeate easily and in solutions permeating with difficulty. The anatomical investigations have shown a point of probable easy permeation, in agreement with COLLINS, SCHROEDER, and R. BROWN, as well as a gradient of thickness of the limiting membranes which is in support of the theories of HARRINGTON and CROCKER, and SHULL. In view of the results presented in this paper, it is entirely probable that not only is there an initial point of rapid permeation (the micropyle) but there is also a gradient of the seed coats to permeation, with the stretching and swelling resultant from the early basal absorption assisting in the progressive apicalward permeation and absorption by the grain. The thinness of the cutin membrane over the embryo, with the resultant decreased resistance of this area to permeation, probably accounts for the greater portion of the early absorption at the germinal end of the barley caryopsis.

The hull-less barleys studied have been found more easily permeable than the hulled varieties. Anatomical features which might be correlated with this increased degree are: thinner outer cutin membranes of the integument (of that portion covering the embryo in particular, see figs. 27–32); thinner membranes across the micropyle; and more areas of the integument in which there is less of the fatty substances in the inner row of cells. Although at times these variations may be partially controlling factors, it is thought more probable that differences in physical and chemical composition of the cutin membranes may be of more significant importance in regulating the degree of permeability. This hypothesis is substantiated by the increased rate of permeation induced by the wet environ-

ment during maturation, there being no alteration of anatomical structure in any of the varieties studied which could be correlated with these induced changes in permeability. It is then apparent that variations in degree of permeability are, at least in part, dependent upon the composition of the cutin membranes of the inner integument.

Summary

1. The developmental anatomy and morphology of the barley caryopsis has been discussed and illustrated.

2. The selective semipermeable envelope has been identified as the persistent crushed and cutinized inner integument together with the suberized, resistant tissue of the chalaza.

3. The inner integument has a thin inner cutin membrane and a much thicker outer cutin layer of variable thickness. The thickest portion is over the apex of the kernel near its attachment to the chalaza. It is also very thick over the flanks of the endosperm in the groove, is thicker laterally than anteriorly, and it is thicker apically than proximally. It is very thin over the embryo, its thinnest portion being the area immediately across the grouped cells at the micropyle. The thickness of these membranes was found to vary with the variety studied.

4. Relative permeability of the seed coats has been determined in two ways, by the rate of absorption from aqueous solution, and by the electrical conductivity of seed coat membranes in aqueous solutions of electrolytes.

5. With the use of these two differing methods it has been determined that: varieties exhibit wide differences in degree of permeability (the hulled varieties as a group being less permeable than the hull-less varieties); a wet environment during maturation induces a decreased resistance to permeation; and harvesting prematurely also induces a slightly decreased resistance to permeation.

6. The differences found in relative permeability could not be correlated definitely with any one existent variation in the anatomical structure of the semipermeable seed coat envelopes of the caryopsis. With some varieties, however, the heavier layer of cutin on the integument and the heavier deposits of the fat substance in the

integument cells seem to be correlated with an increased resistance to permeation.

7. The hull-less varieties investigated, as a group, were found more permeable than the hulled varieties but this difference was independent of the presence of the hulls.

The writer expresses his gratitude to Professor G. J. DICKSON, who suggested the problem and has offered much constructive criticism during the progress of the investigation; to Professors B. M. DUGGAR and ARTHUR J. EAMES, who have been most helpful; and to the National Research Council who awarded the Fellowship which facilitated the completion of the investigations.

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ECOLOGICAL STUDIES ON THE HIGH PLATEAUS OF UTAH

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 465

HELEN DIXON

(WITH THREE FIGURES)

Introduction

The region studied is located mainly in Wayne County, Utah, but it includes parts of Sevier County (in the vicinity of Fish Lake) and of Garfield County (Boulder Mt. on Aquarius Plateau). It lies within a quadrangle extending from 111° to 112° west longitude and from 38° to 38° 45′ north latitude. The altitudinal range is from 5250 feet at Notom to 11,600 feet on the tops of the High Plateaus.

The drainage is into the Colorado River by way of the Fremont River. Physiographically the region belongs to the province designated by the Powell Survey (3) as "the High Plateaus of Utah." Because of its sparse population and its inaccessibility to the ordinary traveler, it is comparatively unknown and is still unspoiled by tourists.

Geology and topography

The geology was first worked out in 1879 by Capt. C. E. DUTTON of the Powell Survey (3). Little scientific work has been done in this territory since then. The Plateau Province of Powell has been above the sea since the close of the Cretaceous. Tertiary lacustrine deposits and lava flows have covered the earlier marine deposits. The present altitude of over 11,000 feet is due mainly to subsequent faulting and uplift.

The highlands considered in this report comprise the southern half of the easternmost of the three belts of Powell's High Plateaus. They include the Fish Lake Plateau with Mts. Marvine, Terrill, and Hilgard, its outliers northeast of it, the Awapa Plateau, Thousand Lake Mt., and the Aquarius Plateau.

Fish Lake Plateau is a small tableland 10–15 miles long by 2–5 miles wide. It reaches a maximum altitude of 11,600 feet. Except

along its southwest margin where it merges with the Awapa Plateau, it is bounded by precipitous lava walls such as the Grass Valley fault-scarp, a 4300 foot cliff on the northwest, and the 2700 foot cliff above the lake on the southeast side.

There is evidence of glaciation during the Pleistocene. The location of the terminal moraines at 9000 feet in Fish Lake Basin and in Summit and Moraine valleys, however, disproves any glacial origin for the lake basin itself, the lake level being 8750 feet. DUTTON (3) found evidence of a former outlet into the Great Basin at the south end of the lake. In the process of uplifting, the basin was so tilted that the drainage was reversed. The water of Fish Lake now joins that of Seven Mile Creek at the north end, and together they flow through the Fremont Reservoir into the headwaters of the Fremont River and thence into the Colorado River.

Fish Lake is a deep body of water 6 miles long and nearly 2 miles wide. Its west wall rises steeply 2600 to 2700 feet to the top of the plateau. The east wall is even more abrupt but not so high, only 1700 feet. Just over its crest are the two Crater Lakes.

The Awapa Plateau adjoining Fish Lake Plateau on the southeast was uplifted with the latter along the Grass Valley fault. Its western front stands 2000 feet above Grass Valley. Awapa Plateau has an area of about 700 square miles. Its surface is a rocky lava slope descending from 9000 feet at the northwest, west, and southwest rims to Rabbit Valley, a crescent shaped depression lying at 7000 feet above the sea. The Fremont River flows through this valley after its descent from the Fish Lake Highlands and deposits here much of its load, forming a rich alluvial plain.

Rising above Rabbit Valley along the concave side of the crescent is Thousand Lake Mt. It too is a remnant of erosion, having been separated from the Aquarius Plateau by the work of the Fremont River and its tributaries. Thousand Lake Mt. is an even smaller table than Fish Lake Plateau, its nearly level lava-capped summit being about 5 miles long and not quite 2 miles wide. To the east and southeast is the great rock desert of the Water Pocket Flexure, while to the south across the Fremont Valley is the Aquarius Plateau.

At the lower end of Rabbit Valley, the Fremont River passes through the Red Gate into a series of canyons and gorges cut

through 2500 feet of brilliantly colored sediments on its 100 mile course to the Colorado River.

High above all of this is the Aquarius Plateau, which lies within the Powell National Forest, the largest forest in Utah. This plateau is roughly L-shaped, 35 miles long by 10–18 miles wide. Its altitude ranges from 10,500 feet on the south and west to 11,600 feet on the east, where it stands 5500 to 6000 feet above the rock desert at its base. The lava cap varies from 1000 to 2000 feet in thickness. Over three-fourths of its margin is bounded by massive cliffs at the bases of which are steep talus slopes. Numerous glacial lakes, cirques, and moraines furnish evidence that the summit and upper slopes have been glaciated.

Both the Water Pocket Flexure and Miner's Mt. are subsidiary domes extending southeast from below the Red Gate and rising to 8250 feet. They have been deeply and intricately dissected by narrow gorges and canyons antecedent to their uplift. Most of the gorges are dry except during a rain; but cloudbursts occasionally bring a head of water 15 to 20 feet high rushing through the washes, destroying all in its path.

The Water Pocket Flexure has been of interest to the mineralogist because of its deposits of copper and uranium; to the paleontologist because of the abundant Triassic material, and to the anthropologist because of the interesting remains of the Fremont culture of the cliff-dwellers found in its canyon walls. Botanically it is of interest as a northern outpost of the Lower Sonoran desert vegetation.

Climate

The latitudinal extent of this area is only three-fourths of a degree, but its altitudinal range is 6000 feet. Figures from the records of the United States Weather Bureau (1) corroborate SAMPSON'S (5) statement that climatic differences due to altitude are intensified by the type of relief features peculiar to Utah. The length of the growing season, the temperature, precipitation, rate of evaporation, wind, all of these vary greatly even in as small an area as that under consideration. In the lowlands the growing season is two or three times as long (180 to 200 days) as on the tops of the plateaus (60 to 90 days). This factor alone would show its effects on the plant cover.

PRECIPITATION.—Although dependent somewhat upon altitude, precipitation is more affected by topography. Loa, at 7000 feet, has less precipitation than St. George at 2880 feet. BOWMAN (2) remarks that most of the High Plateaus are as dry as southwestern Arizona at one-half the altitude. DUTTON (3) and SAMPSON (5) point out that all lands below 7000 feet in the plateau country are deserts, receiving less than 8 inches of precipitation per year.

The zones lying above 7000 feet receive more moisture than the lowland stations. BOWMAN (2) estimates that the Aquarius Plateau receives 24–30 inches. Fish Lake Plateau and Thousand Lake Mt. probably get as much, for their vegetation is similar. SAMPSON gives figures for three of the forest zones. The aspen-Douglas fir zone is the wettest, receiving 25–29 inches per year. The Engelmann spruce zone comes next with 25.9 inches. The western yellow pine zone has an average of only 19 inches. No figures are given for the piñon-cedar zone, but it probably gets about 15 inches per year. SAMPSON found the average monthly precipitation during the summer to be as follows:

Engelmann spruce zone	2.16 inches
Douglas fir-aspen zone	2.44 inches
Western yellow pine zone	1.52 inches

EVAPORATION.—Owing to their higher temperature and lower humidity, as well as to their scantier precipitation, the lowest altitudes have the highest rates of evaporation. There is a gradual decrease up to the aspen zone, where it reaches a minimum. In spite of its lower temperature, there is an increase in the spruce zone over that of the zone below. This is due not only to lower precipitation, but also to higher winds. During the growing season, the aspen-Douglas fir zone has a total wind of 12,500 miles, compared with that of 27,500 miles in the Engelmann spruce zone (9).

Soils

The soils of the region vary widely in their physical and chemical composition. Residual lava soils occur chiefly on the tops of the High Plateaus, and also on the entire surface of the Awapa Plateau. They are usually thin and stony, owing to the resistant character of

the parent rock. Their moisture content therefore is low and their air content high.

Residual sands, gravels, and clays are found below the lavas on all of the plateaus. Sands and clays are most abundant and show contrasting vegetation in the same climatic zone.

Residual limy soils are less common. On the northeast face of Aquarius Plateau at about 7500 feet altitude is a spur of richly calcareous soil derived from gypsum. Growing here, as at Bryce Canyon where the soil is from a very calcareous sandstone, is *Pinus aristata*.

Residual organic soils accumulate best in poorly drained depressions and usually support a meadow vegetation. A vein of peat 2–3 feet below the surface of glacial till in the *Pinus ponderosa* forest indicates a former climate somewhat different from that of the present. Humus layers cover other soils on all vegetated surfaces.

Aeolian soils in the form of dunes are piled against the White Cliff domes on the sandstone ledges and on a miniature scale on the floodplain of the Fremont River. These soils are like the residual sands except that they are less stable.

Alluvial soils are largely under cultivation. They occur most plentifully in Rabbit Valley and in the Fremont Valley at Fruita. Slope wash has helped to fill Summit Valley and the Fish Lake basin. Alluvium is usually deep and level and better watered than surrounding areas. Its vegetation is therefore distinct.

Lacustrine soils are found along the shores of shallow lakes. Like stream-borne soils, they are level, deep, and well watered. In addition they are usually fine and richer in humus although their drainage is poorer.

Glacial drift is restricted to levels above 9000 feet at Fish Lake and perhaps on the other plateaus. Terminal moraines exist at this level.

Talus, because of its coarseness, usually supports only pioneer stages of vegetation. In the montane zone such soil more frequently develops a conifer rather than an aspen forest. Perhaps the crevices where seeds may start are too dark for aspens.

Most of the soils were circum-neutral (pH 6.8–7.2). The meadow soils showed pH 6.8 or above; soil from the Atriplex desert at Notom

tested pH 7.8–8.0; while the mud flats on the shores of the Crater Lakes at 9750 feet had a pH of 7.3.

Vegetation

The various plant zones, like the belts of climate, are more or less dovetailed together. The southern limit of this area seems to be drawn on the tension line between the upper and lower Sonoran zones, for in the arid regions beyond the Red Gate such conditions exist as permit the advance of the vanguard of the lower Sonoran flora into territory which theoretically should be northern desert.

FIG. 1.—Alkali desert below Notom showing greasewood-shadscale association; *Populus fremontii* along the wash. Photograph by S. B. Spira.

Only a few of the southern desert forms have succeeded in climbing over the rim into the Great Basin. The desert areas on the basin side of the divide are occupied by the northern desert formation dominated by *Artemisia tridentata*. Both of these formations are replaced, at least temporarily, on alkali soils by *Sarcobatus vermiculatus* and its associates (fig. 1). Above the desert, in the cooler, less arid zone extending from 6000 to 7500 feet, are extremely open stands of scrub timber. Near Fruita this zone is occupied by the southern semidesert formation consisting chiefly of *Pinus edulis* and *Juniperus utahensis*, with some northern desert species. The cedar-piñon association forms a belt also above the *Artemisia tridentata* zone on

Thousand Lake Mt., Aquarius Plateau, and the extreme rim of the Awapa Plateau above 8000 feet.

The northern semidesert is likewise characterized by the cedar; but instead of by piñon, it is accompanied by *Cercocarpus*. It separates the sagebrush desert from the montane forest on the west face of Fish Lake Plateau.

There are two other associations between the desert and the forest which are probably only subclimaxes here. In some places are nearly pure stands of *Quercus utahensis*. In others a chaparral consisting of *Arctostaphylos platyphylla* is conspicuous. The composition of the scrub forest zone seems to vary somewhat with edaphic conditions.

Where the rainfall is 15 to 20 inches, the slopes not too steep, and the soil well drained, open but practically pure stands of *Pinus ponderosa* occur. These are the first true forests. The trees are tall and straight and not so scattered as in the semidesert. They form the climax association on the northern and eastern slopes of the Aquarius Plateau from 7500 to 9000 feet, and at about 8000 feet on some of the sandy spurs on the east face of Thousand Lake Mt. Isolated groups occur as low as 5000 feet in favorable situations on Miner's Mt. and the Water Pocket Flexure. There are no western yellow pines on either Fish Lake or the Awapa Plateaus.

Above the western yellow pine forest occurs the most mesophytic plant association in all Utah, the montane forest. In this region it is best developed from about 8700 to 9500 feet, but may extend far down along mountain streams, even into the piñon zone. The dominant trees are *Pseudotsuga mucronata* and *Populus aurea*. Mixed with them are *Abies concolor* and *Picea pungens*. This formation is well represented on all three of the High Plateaus.

The subalpine forest consists mainly of Engelmann spruce; but in places *Abies lasiocarpa* is abundant. Both aspens and Douglas firs extend well up into this zone. The main stand of the subalpine forest lies between 9500 feet and 11,000 feet on these plateaus. It extends down to 9000 feet in canyons and exists only as scattered bunch forests beyond 11,000 feet.

The alpine meadow formation occupies valleys and depressions at the subalpine level. *Caltha rotundifolia, Polygonum bistortoides,*

Phleum alpinum, and *Pedicularis groenlandica* seem to be constant members of this association. The alpine meadow may represent a permanent climax or a long-time temporary climax. Whichever may be the case, it is a characteristic feature of the landscape in the subalpine zone.

In the open spaces on the summits and high ridges the soil is thin and stony, and because of its good drainage and exposure to high winds, it is dry. Here an alpine scrub made up of prostrate shrubs and minute mat-forming herbs develops. This alpine-desert climax is found on the tops of all the High Plateaus and on the summits of Mts. Marvine and Terrill. It corresponds to the dry tundra of the arctic.

1. Southern desert zone

Desert canyons of Water Pocket Flexure.—Although the upland near Fruita belongs to the piñon zone, the canyons and washes here exhibit a more southern flora, representing the northern extension of the Lower Sonoran. This may be accounted for partly by their lower altitude, but it is due also to the fact that the water courses of the Colorado River system must serve as the highways of migration for the Lower Sonoran flora, since overland passage to the north is blocked by the high plateaus in Arizona. In some respects these canyons in the Capitol Reef resemble those in Zion National Park. Both are carved into the Vermilion and White Cliff sandstones; Zion Canyon is farther south and about 2000 feet lower, and so is warmer and has a longer growing season. In spite of this its climate is less arid than that of the Water Pocket canyons, perhaps because it has been eroded deeper and so is more shaded. However, since the Fremont Valley (fig. 2) is farther from the centers of distribution of Lower Sonoran species, it has fewer representatives from that zone.

Most of the canyons in the Water Pocket Flexure are deep and very narrow. All of them except the Fremont itself and several of its larger tributaries are usually dry. Floodplain studies therefore were confined to the lower end of Spring Canyon, to Pleasant Creek, and to the Fremont River. The last is heavily charged with red silt, but its current is so rapid that most of this is carried on to the Colo-

rado River and only the heavier sand and gravel are deposited, except in a few places behind bars or levees.

Mats of *Halerpestes cymbalaria* seem to have been the first pioneers on the wet sand of floodplains. *Ranunculus* spp. and *Muhlenbergia asperifolia* soon follow. On the dry sand of levees and small dunes, *Abronia elliptica* and *Franseria acanthicarpa* are succeeded by *Salsola pestifer*, *Cenchrus pauciflora*, and *Atriplex canescens*. *Salix lasiandra* is the most common pioneer streamside shrub. *Salix exigua* and other willows may accompany it. *Asclepias incarnata*, *Melilotus alba*, *Cleome lutea*, *Stanleya integrifolia*, *Artemisia tridentata*, and *Chrysothamnus graveolens* grow quite tall along the streams. *Clematis ligusticifolia* festoons many of the streamside shrubs. Each zone seems to have its own species of rose. Here it is *Rosa puberulenta*. In sunny places on sandy floodplains *Populus fremontii* follows the willows, although it is sometimes the pioneer tree, as on the floodplain of Pleasant Creek in the desert below Notom (fig. 1). Above the Water Pocket Flexure along the same stream *Populus trichocarpa* and escapes of *P. alba* are common.

On shadier floodplains, *Acer interior* replaces the Fremont cottonwood. With it is *Populus angustifolia* and sometimes *Toxicodendron rydbergii*. In the still denser shade of narrow canyons *Cornus stolonifera* and *Betula fontinalis* grow on the banks of the streams. These have come down stream from a zone or two above. *Fraxinus anomala* and *Rhus utahensis* usually occur abundantly back from the water's edge on the higher floodplain.

As the canyon is widened and the floodplain built up, the former streamside zone becomes more arid, both as to soil and to atmosphere. Its vegetation then retrogresses to the desert formation which is the climax of the region. So on the higher grounds of wide floodplains various cacti (*Opuntia* spp., *Echinocactus*, *Echinocereus*), *Ephedra viridis*, *Yucca harrimaniae*, *Lepargyrea rotundifolia*, *Wyethia scabra*, *Coleosanthus microphyllus*, *Chrysopsis* spp., and the desert grasses *Hilaria jamesii* and *Sitanion hystrix* are typical. Wherever the dry sand is piled up into dunes, *Abronias* and their associates come in.

GRAVEL WASHES.—In the gravel washes where the soil is moister, deeper, and more level than that on the uplands, the vegetation presents a more desert-like aspect than that of the ridges above.

FIG. 2

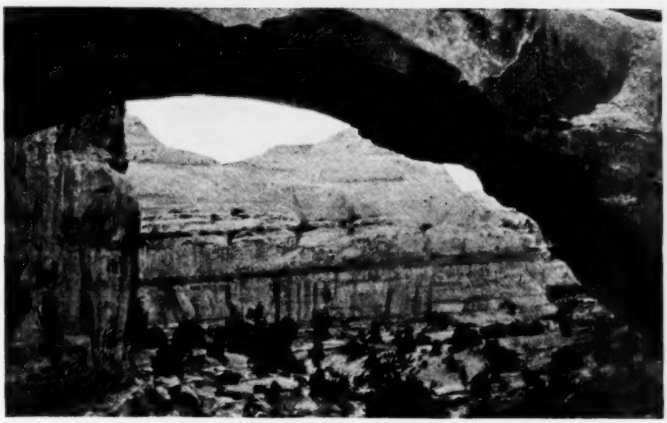

FIG. 3

FIGS. 2, 3.—Fig. 2 (above), Fremont valley above Fruita. The alluvial soil is irrigated for cultivation. The southern desert formation occupies the lower slopes; the cedar-piñon woodland, the upper slopes. Fig. 3 (below), through the arch of the Wayne County (Utah) natural bridge. Cedar-piñon climax on talus of White Cliff sandstone.

Although they are usually dry, a cloudburst every few years is sufficient to sweep the washes clean of all vegetation. Shrubs and herbs may return in the time intervening between wet years, but not such slow growing forms as cedars and piñons. In the draws are *Cleome lutea*, *Polygala subspinosa* (?), *Lavauxia howardii*, *Sphaeralcea grossulariaefolia*, *Phacelia corrugata*, *Delphinium scaposum*, *Astragalus thompsonae*, *Chamaesyce fendleri*, *Asclepias cryptoceras*, *Eriogonum alatum*, and several cacti. Shrubby forms such as *Ephedra viridis*, *Cowania stansburiana*, *Odostemon fremontii*, *Amelanchier utahensis*, *Pleiacanthus spinosus*, *Rhus utahensis*, *Artemisia tridentata*, and *Chrysothamnus graveolens* follow the herbs. In shady places *Quamoclidion froebelii*, *Pentstemon caespitosus*, *Datura meteloides*, *Asclepias labriformis*, *Artemisia filifolia*, *Jonesiella asclepiadoides*, and others are found instead.

ROCK CANYONS.—Higher upstream the sandy deposits are limited to deltas or bars at the mouths of side canyons. Vegetation on the rock floors differs from that of the floodplains. In shady places *Odostemon fremontii* is common. *Juniperus utahensis*, *Pinus edulis*, and *Cercocarpus intricatus* are occasionally seen on the ledges of the canyon walls. On sunny rocks *Ephedra torreyana*, *Lepargyrea rotundifolia*, and a shrubby *Eriogonum* appear. Near a spring at the mouth of a side canyon is a clump of *Celtis rugulosa*.

Narrow canyons develop more mesophytic vegetation. Both western yellow pine and Douglas fir, with a rank undergrowth of *Odostemon repens*, *Ceanothus fendleri*, *Amelanchier utahensis*, *Pentstemon eastwoodii* var. *undosus*, *Selaginella mutica*, and *Phlox austromontana* are found. A colony of *Pinus ponderosa* accompanied by a thicket of *Arctostaphylos platyphylla* occurs on a north-facing sandstone cliff in the lower piñon-cedar zone. The presence of crustose and foliose lichens and xerophytic mosses on white sandstone indicates that erosion is slow.

2. PIÑON ZONE (SOUTHERN SEMIDESERT ZONE)

The nut pine-cedar association is the climax of the southern semidesert zone (fig. 3). It is reached mainly through a xerarch or occasionally through an alkali succession, for there are no ponds or lakes, and the stream beds at this level are dry except when it rains. Stabil-

ity and depth of soil are the main factors in the xerarch succession in this zone, for even in the climax pine-cedar woodland the plants are spaced so far apart that light and moisture conditions are not very different from those of the pioneer stages. The tree stages, however, do require a somewhat deeper soil. The type of soil does not seem to be important, for this southern semidesert formation occurs on white sands, red stony clays, and on heterogeneous soils derived from conglomerates containing huge black lava boulders. *Acarospora* and other crustose lichens initiate the succession on the lava. They are followed by *Parmelia* spp. and the moss *Tortula muralis*. Shale and sandstone weather so rapidly that these earliest stages are usually omitted on them. Crevice plants such as *Selaginella mutica* are more commonly the first pioneers on the sandstone ledges. In the deeper crevices almost any species of the piñon-cedar association, including the dominant trees themselves, may be found. Pioneer crevice herbs aid in enlarging the small pockets and accumulating sufficient soil for shrubs and eventually for trees. The wind, too, is an important agent in this succession, for it honeycombs the sandstone walls (fig. 3) and then piles the sand on the ledges in the form of dunes. On these dunes the pioneers are of a different sort. They must have root systems which can aid in binding the shifting sand or which can endure a fluctuating soil level. *Abronia elliptica*, *Franseria acanthicarpa*, *Salsola pestifer*, *Arenaria aculeata*, *Townsendia arizonica*, *Hilaria jamesii*, *Erigeron argentatus*, *Gilia dixonae* Nels., *Phlox canescens*, and several species of cacti are dune pioneers. *Odostemon fremontii* and *Rhus utahensis* represent the shrub stage. The trees are less common on dunes than in large crevices or in rocky soils where anchorage is better.

The herbs and shrubs are much more abundant and the trees are closer together, larger, and more symmetrical on coarse rocky soils than on the wind-polished sandstone plateaus (fig. 3). The dominant herbs are *Sphaeralcea grossulariaefolia*, *Lygodesmia juncea*, *Calochortus nuttallii*, *Lavauxia brachycarpa*, *Stipa speciosa*, *Aristida fendleriana*, *Andropogon scoparius*, *Artemisia wrightii*, *Pentstemon ophianthus*, *Pleiacanthus spinosus*, *Opuntia* spp., *Echinocactus xeranthemoides*, *Echinocereus triglochidiatus*, and *E. mojavensis*. The woodier forms include *Yucca harrimaniae*, *Artemisia tridentata*, *Chrysotham-*

nus graveolens, *Lepargyrea rotundifolia*, and *Amelanchier utahensis*. *Philadelphus microphyllus*, *Odostemon fremontii*, *Rhus utahensis*, and *Quamoclidion froebelii* are more common on shady slopes. These species perhaps precede the trees, but continue on with them in the richest piñon-cedar woodland.

STREAMSIDE VEGETATION IN PIÑON ZONE.—The permanent streams in this zone are often fringed merely with more luxuriant sagebrush and rabbit brush, but the narrow-leaf cottonwood seems to be the typical streamside species. It may be preceded by willows. *Rosa puberulenta* and *Rhus utahensis* accompany it. *Urtica breweri* is common on the floodplain of the Fremont River. *Acer interior* and *Betula fontinalis* replace *Populus angustifolia* in shaded places. *Picea excelsa* is seen along the streams in the upper limits of the zone.

3. ALKALI ASSOCIATION

On shaly soils alkali crusts may form, for drainage is poor and the salts are not leached out. As at Notom where the pH is 7.8 to 8.0, the piñon-cedar woodland is replaced indefinitely by a long-time temporary climax of *Atriplex confertifolia* and *Sarcobatus vermiculatus*, which may be introduced by *Distichlis spicata* on flats of alkaline mud along the stream. The alkali desert formation (fig. 1) covers the soil very scantily; vast stretches are plantless.

4. NORTHERN DESERT CLIMAX

Almost the entire Awapa Plateau, the west side of Aquarius Plateau, and the lower slopes of the Fish Lake Plateau are covered by the sagebrush formation. The successions here, too, are all xerarch, since there are no ponds, lakes, nor even streams. The lava rock shows the usual pioneer lichen and moss stages. Xerophytic herbs are first in the crevices. They are soon accompanied down at 7000 feet by cacti and yucca, but up at 9000 feet by *Gilia aggregata*, *Pentstemon strictus*, *Calochortus nuttallii*, *Eriogonum piperi*, a bright rose-colored *Opuntia*, and *Lupinus* spp. When the soil layer is deep enough, *Artemisia tridentata* and *Tetradymia canescens inermis* become dominant, but the herbs continue in the interspaces.

Swamps in the sagebrush zone between Richfield and Fish Lake are of the sedge type. Anderson Wash southeast of Sigurd is fringed

with *Salix* spp., *Acer negundo*, *Prunus melanocarpa*, and *Populus angustifolia*. If swamps and streams existed in the sagebrush desert on the Awapa Plateau they would probably have similar vegetation.

5. Northern semidesert

The cedar-mountain mahogany climax occurs on the middle slopes of the west face of the Fish Lake Plateau from about 8000 feet altitude to over 9000 feet on south facing walls. Piñons are dropping out and with them the southern semidesert shrubs, *Lepargyrea rotundifolia*, *Rhus utahensis*, *Fallugia paradoxa*, and *Cowania neomexicana*. Instead of these, *Juniperus utahensis* is accompanied by *Cercocarpus parvifolius*, *C. ledifolius*, *Juniperus scopulorum*, and *Quercus utahensis*, in a mixed woodland in which grow *Artemisia tridentata*, *Purshia tridentata*, *Prunus melanocarpa*, *Amelanchier oreophila*, *Sericotheca diffusa*, *S. microphylla* (?), *Eriogonum effusum*, *Pachystima myrsinites*, and species of *Symphoricarpos* with a rich herbaceous complement of *Lupinus*, species of *Eriogonum*, *Castilleja*, *Pentstemon*, *Solidago bigelovii*, *Gutierrezia*, *Artemisia frigida*, *Agropyron spicatum*, and others. In the lower part of this mountain mahogany zone are *Yucca* and *Opuntia*.

The distribution of these four desert formations seems to be determined by several factors. Temperature may limit the range of the southern desert species. They are most common below 6000 feet in the Fremont Basin. Those requiring long hot summers and mild winters have not yet migrated to the 9000 foot crest of the Awapa Plateau, and so are not included in the desert flora on the Great Basin side of the divide. The southern semidesert occurs in a rather definite belt between 6000 and 7500 feet on the sandstone plateaus, but on lava soils the sagebrush formation occupies this level, and the cedar-piñon zone is pushed up to even 9000 feet in some places. Hence competition, especially with *Artemisia tridentata*, seems to be more important than temperature in limiting the range of cedars and piñons, for prostrate forms of both were found in the subalpine zone on Thousand Lake Mt.

6. Pinus ponderosa climax

In the western yellow pine zone, studies were made of the upland xerarch succession and of the streamside vegetation. The only hy-

drarch succession found was on the tension line between this and the aspen zone. Since the climate in depressions is cooler than that on adjacent ridges, it is included in the account of the aspen rather than in that of the western yellow pine zone.

TIDESTROM'S map (6) shows *Pinus ponderosa* between 7000 and 9000 feet. SAMPSON (5), however, gives its range as 6200 to 7600 feet. On the Aquarius Plateau the main forest lies between 7500 and 9000 feet on the east face and 7000 and 8500 feet on the north face, with isolated colonies as low as 5600 feet. The western yellow pines on the east spurs of Thousand Lake Mt. are at an altitude of about 8000 feet. *Pinus ponderosa* seems to occur here on flat areas of both residual and aeolian sands and on steep slopes of glacial drift. Sandy slopes may be too dry and flat clays too poorly drained for it.

XERARCH SUCCESSION.—On resistant rock, crustose lichens form the initial stages. These are followed by *Parmelia* and other foliose forms, and by xerophytic mosses. After these pioneers, together with agents of erosion, have disintegrated the rock, xerophytic herbs such as *Antennaria aprica*, *Pediocactus simpsonii*, *Opuntia fragilis* (and other *Opuntia* spp. in the lower parts of the zone), *Eriogonum racemosum*, *Bouteloua gracilis*, *Sitanion hystrix*, and *Oreocarya suffruticosa* come in on the new soil. In sandy places *Abronia elliptica*, *Phlox stansburyi*, and *Pentstemon arenicola* are common pioneers. *Pentstemon cyananthus* (?), *Gilia aggregata*, *Linum lewisii*, and *Oreocarya argentea* follow them. *Rosa* spp. are common in loose sand, and *Cercocarpus intricatus* in crevices of sandstone cliffs. In the lower part of the zone *Yucca*, several cacti, and *Pleiacanthus spinosus*, found in the piñon zone, are common pioneers, especially on rocky soils.

Purshia tridentata, *Ceanothus velutinus*, *C. fendleri*, *Amelanchier alnifolia*, *Sambucus melanocarpa*, *Cercocarpus montanus*, *Symphoricarpos oreophilus*, and species of *Ribes*, *Grossularia*, and *Rosa* are fairly common representatives in the shrub stage throughout the zone. *Artemisia tridentata* and *Chrysothamnus graveolens* come in on deeper soils. In the lower part of the zone, *Cowania neomexicana* and *Fallugia paradoxa* extend up from the piñon zone; and in the upper part are such shrubs as *Odostemon repens*, *Arctostaphylos uva-ursi*, and *Prunus melanocarpa*, all common to the aspen zone. Thickets of

Arctostaphylos platyphylla or of *Quercus utahensis* are sometimes seen on sunny slopes. Occasional specimens of *Pinus edulis* or of *Juniperus utahensis* grow taller and straighter here than in their own zone, another indication that competition may be a limiting factor determining their range. *Juniperus scopulorum* is frequent as an understory tree in the western yellow pine forest. Seedlings of *Picea pungens* occasionally come up on open hillsides. The western yellow pine itself comes in when the soil is deep enough and is well drained. It reproduces well, as is shown by the large number (38) of vigorous seedlings in a 10 meter quadrat which had been protected from grazing for five years. In a quadrat of equal size and similar situation but grazed were only nine pine seedlings. Overgrazing leads to soil erosion, and *Pinus ponderosa* seems to require deep soil.

Since this forest is an open one, many of the pioneer forms continue as undergrowth in the climax stage. Certain species, however, are especially characteristic under the pines. *Peltigera canina* grows on the fallen needles. *Phlox austromontana*, a prostrate, awl-leaved, mat-type species with white to clear pink flowers, is perhaps the most conspicuous herb. *Tithymalus luridus*, *Pseudocymopterus montanus*, *Antennaria aprica*, and *Erigeron vetensis* are also common. *Poa pratensis* and *Agropyron spicatum* are dominant grasses. *Clematis pseudoalpina* grows at the bases of tree trunks. On the north face of Aquarius Plateau *Castilleja linariaefolia*, *Geranium richardsonii*, *Hymenoxys odorata*, *Lupinus lepidus*, and a red *Pentstemon* are abundant. *Senecio* and *Hymenoxys* are considered by the rangers as indicative of overgrazing. In places the sheep have practically eliminated the shrubs in the pine forest.

STREAMSIDE VEGETATION OF PINUS PONDEROSA ZONE.—*Salix subcoerulea* and *S. bebbiana* fringe the streams at 8000 feet. The first tree on the floodplain is *Populus angustifolia*. *Betula fontinalis* is a close second. With them on wet banks are gooseberry, elderberry, currant, and rose, accompanied by *Veronica arvensis*, *Juncus* spp., *Artemisia racemosa*, *Montia chamissoi*, *Equisetum hiemale*, mosses, and liverworts. Growing in the stream among rocks are *Cardamine infausta* and *Catabrosa aquatica*. In the next belt back from the stream is *Picea pungens* with *Juniperus scopulorum* and *J. sibirica*. Among these conifers are *Smilacina stellata*, *Geranium richardsonii*,

Arenaria eastwoodii, and *Wyethia mollis*. The willows and cottonwoods seem to be limited to the stream's edge, but the blue spruce has a wider range.

OAK THICKETS.—SAMPSON (5) points out the oak as an ecological equivalent of *Pinus ponderosa* which is capable of replacing the pine. Usually the sheep browse the oak so closely that it remains a low shrub. On steep, rocky, more inaccessible slopes it attains a height of 7–10 feet. Typical pine forest pioneers seem to precede *Quercus utahensis* and a typical pine undergrowth accompanies it: *Odostemon repens*, *Castilleja linariaefolia*, *Phlox austromontana*, *Ceanothus fendleri*, and seedlings of *Juniperus scopulorum*. On Aquarius Plateau the oak association seems to be a pioneer tree stage which precedes the climax western yellow pine forest, and apparently is not an ecological equivalent of it. On the west face of Fish Lake Plateau the oak appears on ridges in the upper part of the mountain mahogany zone at the same level where aspens are first seen in the ravines.

MANZANITA.—*Arctostaphylos platyphylla* also forms only small local colonies here, and so can scarcely be considered as anywhere replacing the pine as a climax form. Instead it seems to occur as a subclimax stage in the xerarch succession to the pine forest.

FOX-TAIL PINE.—Although classified by TIDESTROM as belonging in the aspen-spruce zone, *Pinus aristata* is found here only in two small colonies in the western yellow pine zone on the north face of Aquarius Plateau. In such a situation it may be a relict of the Pleistocene.

7. MONTANE ZONE

This zone is best exhibited on north and east facing slopes in a belt extending from 8700 to 9500 feet in altitude. The aspen forms extensive groves. The Douglas fir is not found in as pure stands as the aspen; it seems to be restricted to the steeper, more exposed slopes, rocky ridges, talus slides, etc., while the aspen occupies morainic lacustrine, or alluvial deposits where the soil is deeper and moist. In shaded mesophytic canyons and about springs on slopes, aspens descend to 7500 feet, and perhaps occasionally lower. The lowest altitude recorded for Douglas fir in this region is about 6000 feet. On the other hand, both species occur mixed with Engelmann spruce and subalpine fir in the forests below the lava caps on all three of the

plateaus. There are dwarf aspens as high as 10,700 feet. As a rule, however, they do not reach the summits.

Like the sagebrush, the aspen takes the deeper, moister soils of pockets and shady north faces in the lower part of its range, but comes out on the ridges in the upper stretches, for below, the ridges are too dry, and above, the summers in the pockets are too short.

The Douglas fir association seems usually to climax a xerarch succession, but both xerarch and hydrarch series may terminate in the aspen association.

XERARCH SUCCESSION TO CLIMAX MONTANE FOREST.—Wherever resistant rock is found, the earliest stages are the usual crustose lichens, *Acarospora bella*, *A.* spp., *Caloplaca* spp., and *Candelariella vitellina*, followed by *Parmelia molliuscula*, *P. conspersa*, and the moss *Tortula muralis*. The succession on soil begins with xerophytic herbs, the most conspicuous of which are *Pleiacanthus spinosus*, *Artemisia frigida*, *Chaenactis douglasii*, and *Pediocactus simpsonii*, this last a cactus which seems to be limited to moderately high altitudes (8700–9800 feet) and to thin rocky soils. Other xerophytes accompany these pioneers later and eventually crowd them out: *Erigeron bloomeri*, *E. flagellaris*, *Antennaria aprica*, *Eriogonum racemosum*, *E. jamesii*, *Castilleja exilis* and other species, *Lupinus argenteus*, *L. alpestris*, *Sitanion hystrix*, *Stipa comata intermedia*, *Koeleria cristata*, *Gilia aggregata*, *Phlox cortezana*, *Senecio multilobatus*, *S. mutabilis*, and others. These herbaceous xerophytes form the present cover on the dry knolls of the spurs projecting from the façades of the plateaus. Below them is a shrub zone which dovetails into the aspen grove or Douglas fir forest, as the case may be. The xerophytic shrub pioneers are common to the pine forest below. They include *Tetradymia canescens inermis*, *Artemisia tridentata*, *Purshia tridentata*, species of *Chrysothamnus*, *Amelanchier alnifolia*, *Sambucus melanocarpa*, *Rosa fendleri*, *Ceanothus fendleri*, *Ribes leptanthum*, *R. viscosissimum* (?), and *Prunus melanocarpa*. Just preceding the aspen is *Symphoricarpos oreophilus*, which remains as the dominant undershrub in the aspen grove. *Lepargyrea argentea*, *Odostemon repens*, *Lonicera involucrata*, *Arctostaphylos uva-ursi*, and *Pachystima myrsinites* form the undergrowth in the upper parts of the zone.

No doubt the aspen first establishes itself in any given place by

seeds; but the young aspens seen are practically always adventitious shoots which seem gradually to close in on these dry hilltop meadows until at last the ridge is completely covered by them. In these pure stands the shade is dense in summer, so the soil is moister than in the preceding stages. Consequently a new association of herbs replaces those of the dry meadow. *Geranium richardsonii*, *Castilleja lauta*, *C. arcuata* (?), *Aquilegia coerulea* and its variety *albiflora*, *Arabis drummondii*, *Phacelia sericea*, *Achillea lanulosa*, *Microseris* spp., *Fragaria glauca*, *Senecio atratus*, *Vicia americana*, and a *Delphinium* are found. In many of the upper aspen groves there is a ground cover consisting of an almost pure stand of *Lathyrus leucanthus*. *Odostemon repens* and *Pseudocymopterus montanus* are also common in the high aspen groves.

In wide areas on these plateaus are absolutely pure stands of aspen, the trunks often reaching 18 inches in diameter. On some slopes seedlings of Douglas fir, white fir, blue spruce, and (higher up) subalpine fir and Engelmann spruce are abundant under the aspens. In these mixed stands the aspens are the largest and dominant trees. This may be due to their more rapid growth, to their greater resistance to fire, or to their general freedom from timbering.

The Douglas fir never stands alone, but it dominates certain rocky slopes. The stages leading to this mixed Douglas fir forest are in general the same as those preceding the aspen, but not so rich in species. Since the Douglas fir succession is more apt to take place on a rocky slope, the pioneers consist of lichens and mosses on the rock faces with crevice plants between them.

The common shrubs are *Odostemon repens*, *Amelanchier alnifolia*, *Ribes viscosissimum*, *Ceanothus fendleri*, *Sambucus melanocarpa*, *Artemisia frigida*, *Symphoricarpos oreophilus*, *Arctostaphylos uva-ursi*, and *Pachystima myrsinites*. This succession differs from that leading to the aspen in that pioneer trees (*Cercocarpus montanus*, *C. ledifolius*, *Picea pungens*, *Pinus edulis*, *Juniperus scopulorum*) sometimes precede the climax forest. The blue spruce, which is often thought of as a streamside tree, is common here on dry rocky slopes with the Douglas fir. A forest island of *Picea pungens* in the midst of a dry meadow of cactus, sagebrush, etc., was found on a lava ridge at 9850 feet.

The shade under Douglas firs and other conifers is denser than that formed by the aspens; hence the ground cover is much less luxuriant. Most of the shrubs disappear as the Douglas fir grows up. *Odostemon repens*, *Juniperus sibirica*, *Symphoricarpos oreophilus*, and *Rosa pyrifera* endure the shade. *Lathyrus leucanthus*, *Pseudocymopterus montanus*, *Poa fendleri*, *Festuca ovina*, *Aquilegia flavescens*, *A. coerulea albiflora*, *Oxytropis deflexa*, and *Fragaria bracteata* are herbs of the Douglas fir forest. On the whole, the Douglas firs form only medium sized trees in this area. Specimens up to 30 inches in diameter at 4 feet above the ground are sometimes seen.

HYDRARCH SUCCESSION TO CLIMAX MONTANE FOREST.—The hydrarch succession may be found in ponds or in spring swamps which are located where ground water issues from the lava talus above the glacial clay. The soil of these spring banks was the most acidic of any of the samples tested, its pH varying from 6.4 to 6.6. The streamlets are fringed with a lush vegetation consisting of hydrophytic mosses (*Philonotis fontana*, *Aulacomnium palustre*, etc.) and the herbs *Mimulus guttatus*, *Aconitum columbianum*, *Habenaria elegans*, *H. unalaskensis*, *Galium boreale*, *Sidalcea candida*, *Hypericum formosum*, *H. scouleri*, *Polemonium viscosissimum*, *Thermopsis montana*, *Carex festiva*, *Juncus saximontanus*, *J. balticus*, *Chamaenerion angustifolium*, *Veronica alpina*, *Epilobium ovatifolium*, *Geranium richardsonii*, and *Mertensia pratensis*. The pioneer shrubs are *Salix subcoerulea*, *S. bebbiana*, and other willows; they are followed by *Rosa woodsii* and *Lonicera involucrata*. Blue spruce is the pioneer tree in wet soil. Both aspens and Douglas fir seedlings occur on these spring banks, but the former are more common.

There are few aquatics in the deeper lakes such as Fish Lake, for their water is too cold. A gelatinous alga, *Chara*, *Myriophyllum spicatum*, and species of *Potamogeton* represent the submerged stage. *Batrachium trichophyllum*, *B. grayanum*, *Polygonum amphibium*, and *Hippuris vulgaris* surround the central zone of *Potamogeton americanus* in shallow lakes. These amphibious species are usually succeeded by a wet meadow which is often introduced by an almost pure stand of *Eleocharis acicularis*. *Roripa sinuata* and a small *Ranunculus* are sometimes first. Next on flat shores come *Eleocharis palustris*, *Juncus balticus*, *Alopecurus aequalis*, *Halerpestes cymbalaria*, and

Myosurus aristatus—a small plant usually found on alkali mud at 4000 to 5000 feet in the Great Basin, and seemingly out of place on the shores of mountain lakes at 9700 feet. Next on the moist mud comes a zone of *Rumex mexicanus* with mats of *Taraxia breviflora*. A sedge meadow follows with *Phleum alpinum*, *Deschampsia caespitosa*, *Poa ampla*, *Agropyron tenerum*, *Stipa columbiana*, *Hordeum nodosum*, *Epilobium adenocaulon*, *Thermopsis montana*, *Antennaria microphylla*, *Arnica ocreata*, and species of lupine mixed with the numerous species of *Carex*.

Higher up is a drier meadow of *Dasiophora fruticosa*, *Pentstemon procerus*, *Artemisia cana*, *A. trifida*, *Achillea lanulosa*, *Pedicularis parryi*, *Potentilla pulcherrima* (?), *P. propinqua*, *Rosa pyrifera*, etc. Where the shores are steep and rocky instead of flat and muddy, narrow amphibious zones may be followed immediately by a fringe of willows or even of aspens.

STREAMSIDE VEGETATION.—In the open, swampy meadows occur along pre-erosion streams. Here hydrophytic sedges, mosses, and rushes are accompanied by clumps of willows, colonies of *Iris missouriensis*, *Carex nebraskensis*, *C. lanuginosa*, *Koeleria cristata*, *Oryzopsis hymenoides*, *Sisyrinchium angustifolium*, *Valeriana edulis*, and *Equisetum hiemale* followed by seedlings of blue spruce and later by aspen. In the higher places *Castilleja exilis*, *Agropyron tenerum*, *Aira caespitosa*, *Potentilla pennsylvanica*, *Aplopappus uniflora*, *Antennaria microphylla*, *Hypericum formosum*, rose, currant, and gooseberry come in.

Where the pre-erosion streams flow through the forest instead of a meadow their vegetation is similar to that of the spring banks. Among the rocks in forest streams grow *Cardamine infausta*, *C. cordifolia*, *Mimulus guttatus*, and *Epilobium ovatifolium*. *Aulacomnium palustre*, *Philonotis fontana*, *Conocephalum* sp., and *Marchantia* sp. border the streams. Among them are tufts of *Moneses uniflora*, *Pyrola chlorantha*, *Saxifraga rhomboidea*, *Smilacina stellata*—mostly representatives of zones higher up which are adapted to the cool water of these mountain streams. Wherever there are openings in the forest the willow fringe is seen, usually *Salix subcoerulea* and *S. bebbiana*. *Alnus tenuifolia* may be with them. Blue spruce seems to be a universal streamside tree of this zone, both in the open and in

the forest. With it are *Filix fragilis, Galium boreale, Phacelia idahoensis, Geranium richardsonii, Aconitum columbianum, Delphinium subalpinum, Smilacina stellata, Pyrola asarifolia, Veronica wormskjoldii, Epilobium adenocaulon, Phleum alpinum, Hypericum formosum, H. scouleri, Castilleja lauta, Polemonium foliosissimum, Habenaria elegans,* and *H. unalaskensis.*

In the few places where erosion has begun *Equisetum hiemale* is common on clay or drift banks above the line of present stream action. On new soils of small floodplains mats of *Halerpestes cymbalaria* are first; then willows, alders, and blue spruces occupy the stream deposits. Back farther on drier flats are raspberry, gooseberry, currant, rose, and sometimes *Juniperus sibirica* with *Poa canadensis, Fragaria bracteata, Potentilla glaucophylla, Phleum alpinum, Mertensia pratensis, Viola nephrophylla,* dandelions, and yarrow. Aspen comes in on the older floodplain accompanied by the climax shrubs such as *Symphoricarpos oreophilus, S. longiflorus, S. racemosus, Odostemon repens,* and the herbs of the aspen stage.

MONTANE CLIMAX FOREST.—The fact that aspen terminates both xerarch and hydrarch successions might seem to indicate that this tree is the true climax here. Its edaphic distribution would also class it as more mesophytic than *Pseudotsuga.* Yet the aspen does not seem to have a well defined undergrowth. Possibly the inconstant character of its undergrowth indicates that the aspen is not a true climax, as has been suggested by some, but a long-time temporary climax, as others contend. Perhaps it may be merely a stage in a secondary succession following fire, as some of the rangers think; but there were few signs of past burns. The aspen may be found succeeding itself, forming dense pure stands. It does so, however, by means of adventitious shoots, not by seeds germinated in its own shade. The aspen may be seen also sheltering seedlings of Douglas fir, or it may come in simultaneously with Douglas fir, or even after it. Both trees extend up into the Engelmann spruce zone; but the aspen is far more abundant than is the Douglas fir. The latter extends lower into the piñon zone than does the aspen, so it must be more tolerant of dry climate. The aspen is clearly more advanced as to soil requirements. On the other hand, aspen seedlings are more light-tolerant; but it colonizes mostly by means of adventitious shoots, and

these do well in shade. Thus this species, like the redwood, is able to maintain itself in conditions of extreme mesophytism although it ranks as a pioneer with respect to its light tolerance. The question whether the aspen or the Douglas fir is the true climax tree of this zone or whether they are ecological equivalents is still an open one.

8. Subalpine forest zone

Overlapping the upper 500–1000 feet of the montane forest, and reaching up to the summits is the subalpine forest. This zone, extending in the main from 9500 to at least 11,000 feet, is characterized by low annual temperatures, a moderate amount of moisture, a short growing season, and high winds. Snow falls as late as June and remains well into July, and the next cold season begins again with snow as early as the last of August.

Without doubt the dominant tree of this association is the Engelmann spruce. It extends from altitudes of 9000 feet at the bases of north facing canyon slopes up to the summits of the plateaus. From 9500 feet up it is the most abundant tree species, and in certain situations the only one. It has unfortunately fallen prey to the ax and the beetle; but tall, straight specimens with trunks 4 feet in diameter are still not uncommon in the dense forest belt just below the lava caps of the plateaus. On the summits and on the rock ridges, where the soil is thin and the wind is strong, it is of course stunted and gnarled; in such places it occurs only in small forest islands.

From 9500 feet up to the base of the lava cap, the Engelmann spruce is accompanied as usual by *Abies lasiocarpa*. The firs here have a narrower range than have the spruces, for neither do they extend as low on the north facing slopes nor do they reach the plateau summits. On south faces their range is from 9800 to about 10,700 feet, the highest altitude noted here for the subalpine fir. The altitudinal limits of this timberline tree seem to be restricted by edaphic rather than by climatic conditions; for wherever the soil is deep, as on the moraines or around alpine lakes, the fir is found. But on the thin, rocky residual lava soils on the summits, the Engelmann spruce stands alone. Throughout the zone the fir is only a subordinate species.

The aspen and the Douglas fir of the montane forest, which are

thoroughly mixed with the spruce and the fir in the lower part of the zone, thin out above. At 10,700 feet the last dwarfed and gnarled aspens were seen just leafing out on June 21 in 1932. Ranger Peterson of Elkhorn Ranger Station reported some limber pines on the summit of Thousand Lake Mt., but I did not find them. At 9800 feet are prostrate specimens of piñon and cedar 1 foot or so in diameter, growing with Douglas fir, aspen, Engelmann spruce, and subalpine fir. TIDESTROM (6) gives 6000 feet as the altitudinal limit of the piñon and RYDBERG (4) locates it at about 8000 feet. Only the yellow pine is lacking at this site to make a complete series.

The subalpine zone is well represented on all three plateaus. Both xerarch and hydrarch successions were found.

XERARCH SUCCESSION TO SUBALPINE CLIMAX.—Xerarch successions are best seen on talus slides and on lava cliffs. On the bare rock are four or five species of crustose lichens: *Acarospora bella*, a black *Acarospora*, *Caloplaca* spp., and *Candelariella vitellina;* semifoliose species such as *Lecanora rubina* and *L. saxicola* follow the xerophytic mosses such as *Tortula muralis* and possibly *Hedwigia*. *Selaginella watsoni* comes in in crevices and on the moss cushions. These early pioneers are followed on rock by the same herbs which introduce the succession on glacial soils and in crevices. On vertical lava walls, *Monardella odoratissima* is nearly always found wherever a little soil has collected. *Polemonium confertum*, *P. pulcherrimum*, *Draba aurea*, *Filix fragilis*, and *Oxyria digyna* are also found in rock crevices in this zone. On deeper soil which is sheltered from the wind, *Mertensia pratensis*, *Polemonium pulcherrimum*, *Aquilegia coerulea*, *Drymocallis fissa*, *Eriogonum subalpinum*, *Erigeron flagellaris*, *Antennaria rosea*, *Phacelia sericea*, *Synthyris laciniata*, *Anemone globosa*, *Sieversia ciliata*, *S. turbinata*, and *Potentilla glaucophylla* are common. In moist crevices *Primula parryi* grows luxuriantly. Where grazing is heavy *Achillea lanulosa*, *Taraxacum officinale*, *Hymenoxys richardsonii*, and *Dugaldia hoopesii* come in. Shrubs follow the herbs. *Dasiophora fruticosa*, *Sambucus microbotrys*, *Ribes lentum*, *Rubus strigosus*, and *Juniperus sibirica* are most frequent; but *Ribes cereum*, *Shepherdia canadensis*, *Lonicera involucrata*, *Arctostaphylos uva-ursi*, *Pachystima myrsinites*, and *Odostemon repens* are also found, especially in the lower parts of the zone. In the shelter of boulders on the summits,

Juniperus sibirica and *Ribes lentum* present compact wind-carved thickets in the lee of which the Engelmann spruce starts. The spruce, too, is dwarfed and twisted. One specimen with a trunk 2 feet in diameter was only 18 feet tall. The spruce islands on the summits retain a border of the herbs and shrubs just listed.

Below the rim where the wind is checked the trees become tall and symmetrical. They form continuous forests which cast a shade so dense that the pioneer shrubs and herbs are eliminated. As undergrowth are *Arnica cordifolia*, which may be considered as an indicator of this association, *Saxifraga rhomboidea*, *S. arguta*, *Pyrola secunda*, *Pseudocymopterus montanus*, *Lathyrus leucanthus*, *Thalictrum megacarpum*, *Fragaria bracteata*, *Peltigera* sp., and a *Castilleja* which seemed to be the earliest species through the snow. A species of *Usnea* and *Letharia divaricata* sometimes festoon dead limbs of the spruce. The understory in the climax spruce-fir forest is very sparse, except where there are openings in the forest canopy, for not only is the shade too dense for most plants, but it prevents early thawing of the snow, so that the season is considerably shortened as compared with that of similar situations in the open. Fleshy fungi are frequently abundant on the humus in these forests. Such forms as *Morchella*, *Boletus*, *Clavaria*, and *Lycoperdon* are plentiful.

HYDRARCH SUCCESSION TO SUBALPINE CLIMAX.—Depressions in this zone such as ice-scoured basins, morainic hollows, or troughs dammed up against the lava wall by glacial deposits are occupied by lakes or meadows and surrounded by the subalpine forest. Whether the hydrarch succession attains a forest stage or terminates in the alpine meadow is a question. But whether the alpine meadow is a true climax or merely a long-time temporary climax, it seems to occupy all of the situations which ever have been hydrophytic. In some places the forest is bordered by a belt of Engelmann spruce seedlings which seem to be encroaching upon the meadow surrounding the lake; but usually there is a sharp line between the forest and the meadow, the latter occupying the sediments in the depressions and the former the rocky shores of the basins.

Numerous bodies of water varying from a few feet to a mile or so in diameter are found between 9500 and 10,000 feet. Those at lower altitudes are bordered by the subalpine forest on their north and

east facing shores, but by the montane forest on the south and west faces. In the higher, deeper, and consequently colder lakes there are no submerged or floating aquatics, but near the shores there may be a zone of rushes (*Juncus* spp.) followed by a zone of sedges (*Carex* spp.) in shallow water. In the free water of shallow ponds, however, grow *Hippuris vulgaris*, *Potamogeton americanus*, and *Polygonum amphibium*. Following the sedge zone on flat shores is a meadow of gentians (*G. heterosepala*, *G. plebeja*, and others), mosses (*Bryum capillare*, *Aulacomnium palustre*, *Climacium americanum*, *Mnium serratum*, etc.), and *Castilleja* spp., *Equisetum hiemale*, *Sagina saginoides*, *Arenaria sajanensis*, *Taraxia breviflora*, and species of *Antennaria*. One lake at the base of the Thousand Lake lava is surrounded by a slimy white mud on which grows *Leontodon scopulorum*, the dwarf dandelion with heads not more than a quarter of an inch in diameter, on scapes less than 1 inch tall.

On steep shores, which are more common, is a narrow fringe of willows at the water's edge. These are followed closely by subalpine shrubs, *Juniperus sibirica*, *Ribes roezli*, *R. niveum*, etc.; then by aspens with an undergrowth of *Fragaria bracteata*, *Geranium richardsonii*, etc.; and these in turn by the Engelmann spruce and the subalpine fir, which come almost to the water's edge, yet do not seem to occupy any zone which has been a part of the lake itself.

Some ponds are developing a sedge fen with a belt of *Zygadenus elegans* around it.

In the deep valleys, or even in only slight depressions on the tops of the plateaus, there develop alpine meadows which have the appearance of permanent features interrupting the forest and giving the landscape the parklike aspect of the Kaibab Plateau in Arizona.

STREAMSIDE VEGETATION IN SUBALPINE ZONE.—Most of the streams in this zone issue from snow banks or from ponds on the summit and are yet of the pre-erosion type. Increased soil moisture along their courses is the main alteration they make in the subalpine forest. Where they are wide enough they eliminate a band of trees through the forest, thus increasing the illumination at the herb level. Among the rocks in these streams are *Cardamine infausta*, *C. cordifolia*, and *Primula parryi*. On moist sunny banks is a rich growth of *Aconitum columbianum*, *Delphinium cockerellii*, *Mertensia pratensis*,

Allium brevistylum, *A. cernuum*, *Sium cicutaefolium*, *Aquilegia coerulea*, *Arnica cordifolia*, and the mosses *Amblystegium serpens*, *Swartzia montana*, *Polytrichum juniperinum*, *Bryum schleicheri* (?), *Brachythecium idahense*, *Mnium serratum*, *Pohlia cruda*, and *Pohlia nutans*. Several species of *Castilleja* and *Lupinus* also occur. *Pentstemon whippleanus* is found along practically all streams in the subalpine forest but almost nowhere else.

When the streams flow through open meadows, they often have a willow fringe, *Salix subcoerulea*, *S. bebbiana*, and others. The meadows are in more level places than the forests, so the streams are more sluggish through them. In the wet mosses are *Viola macloskeyi* (?), *Gentiana heterosepala*, *G. plebeja*, *Parnassia parvifolia*, *Ranunculus inamoenus*, *R. calthaeflorus*, *Moneses uniflora*, *Pyrola chlorantha*, *Carex engelmannii* and other herbs, and *Ribes cereum* and *R. lentum*.

No eroding banks nor floodplains were found along the streams in this zone.

CLIMAX STAGE OF SUBALPINE ZONE.—The montane forest is often described as the finest of the mountain forests. On these plateaus the western yellow pine climax, although more open, exceeds it in the size of the individual trees, and the subalpine forest exceeds it in both density and size of trees. Where it is protected from high winds, the Engelmann spruce forest gives the impression of greater mesophytism even than the aspen groves below it. Since the Aquarius is the largest of these plateaus, it is less affected by wind and so has more extensive areas of these fine forests. In the past 15 years, nine-tenths of the adult Engelmann spruces on Boulder Mt. have been destroyed by a bark beetle, *Dendroctonus engelmannii*. The young spruces are not attacked because they are too resinous. Those below the rim seem to have been bothered less than those on the summit. The moisture conditions may account for this difference, since more vigorous growth results in greater production of resin. Climatic cycles may also be a part of the complex, since a series of dry years would give the beetle the advantage while a wet series would enable the forest to combat it.

Since the Engelmann spruce is the sole tree species on the top of the plateau, the next step in the vegetational history is conditioned

by the question, Will the beetle wipe out the forest entirely? It has nearly succeeded on Boulder Mt. at least. Or, will young trees grow up rapidly enough to replace the old ones destroyed? The spruce bears cones while still young and resinous enough to ward off the beetle. Will these young trees remain immune until the beetle is starved out, or will they mature rapidly enough to keep it supplied with food? If the forest is destroyed, what next? Will the entire plateau top develop as a meadow? Or, will another subalpine tree replace the spruce? The subalpine fir is not bothered by this beetle, but it is only the subordinate tree of the subalpine forest here. It has not succeeded so far in establishing itself on the summit. Douglas fir reaches timber line elsewhere, but it, too, is more or less scarce here. The few aspens which have reached the summit are dwarfed and gnarled. Conditions are too hard for them. Elsewhere some forest successors are usually ready to replace the fallen species.

9. Alpine meadows

In the alpine and subalpine zones the depressions usually seem to give rise to a mesophytic meadow which may represent a climax alternating at such altitudes with the spruce forest, or it may represent simply a long-time temporary climax which will eventually give way to the spruce forest. As long as these areas are basins and valleys, they will have a colder and moister climate than the ridges adjoining them. Because of late-lying snows their growing season is shorter. Because of protection from wind their evaporation is less. Because of higher water-table their soil moisture is greater. And because of cold-air drainage into them their temperatures are lower. In short, their climate is not that of the neighboring forest. If every climate has its climax, we should not expect the same climax in these two situations.

Some of the same species found in these mountain meadows are typical also of snow bank vegetation. Around snow banks on slopes we get much the same climatic conditions—short season, low temperature, and high moisture. Plants found in such places must be tolerant of such conditions, although they may be unable to compete elsewhere with other species. Probably not until the depressions are filled so that their climate approaches that on the slopes

surrounding them will conditions allow the forest to replace the meadow.

The depressions on the summit have thinner soil and are shallow and therefore windier than those in the zone below. The dry alpine scrub association eventually supplants the wet meadow species in these places. Below the subalpine zone where the season is longer, the forest may replace the meadow. In both of these situations the mesophytic alpine meadow association is but a subclimax.

In this plateau region the typical meadow formation occurs in valleys and other depressions from 9000 to 11,000 feet and around snow banks on the summits. Small pre-erosion streams make their way through many of them. The alpine meadow here is apparently always reached through a hydrarch succession, which is one of the arguments for considering it but a temporary climax.

Deep water at such altitudes is too cold for vegetation, but in shallow water, rushes (*Eleocharis acicularis*, *Juncus filiformis*, *J. saximontana*), sedges (*Carex ebenea*, *C. siccata*, *C. variabilis*, and others), and aquatic mosses (*Amblystegium riparium* vars. *fluitans* and *longifolium*, *Crataneuron filicinum*, *Bryum caespiticium*, *Drepanocladus aduncus intermedius*, *D. uncinatus*, *Mnium affine rugicum*, and *Philonotis fontana*) appear as the hydrophytic pioneers. Some meadows are occupied by an almost solid stand of this association. Such a spongy turf seems to develop most frequently in silty meadows in this zone. Streams running through the sedge fen are bordered by *Salix pseudomyrsinites aequalis* (?), *S. subcoerulea*, and other willows.

Rocky floors of valleys do not seem to be favorable for the formation of a sedge turf. Instead they usually develop the typical mountain meadow association. Summit Valley at 9500 feet is a good example. Here the pioneer aquatic rushes and sedges are followed first by *Caltha rotundifolia*, which often pushes its flower stalks through the snow. Soon after it comes *Polygonum bistortoides* and *Pedicularis groenlandica* or its variety *surrecta*. These three species and *Phleum alpinum* are always to be found in the mountain meadows of this region. *Deschampsia caespitosa*, *Poa longiligula*, *Gentiana heterosepala*, *G. plebeja*, *G. calycosa*, and *G. affinis* may also be found in the wet meadow zone. Next comes in a somewhat drier belt of such

forms as *Zygadenus elegans*, *Ranunculus calthaeflorus*, *Clementsia rhodantha*, *Dodecatheon pauciflorum*, *Veronica wormskjoldii*, *Saxifraga rhomboidea*, *Sieversia brevifolia* (?), *S. ciliata*, *Trifolium rydbergii*, *Poa sandbergii*, *Thermopsis montana*, *Potentilla effusum*, *P. concinna*, *P. monspeliensis*, *Lupinus parviflorus*, *Antennaria aprica*, *A. parvifolia*, *Senecio petrophilus*, and several species of *Castilleja* (*C. lauta*, *C. arcuata*, and others). This stage probably represents the climax of this series. In badly overgrazed meadows the climax forms may be partly or wholly replaced by such weedy species as *Hymenoxys richardsonii*, *Antennaria aprica*, *Androsace diffusa*, *Orthocarpa lutea*, yarrow, and dandelion. Along the streams are *Delphinium subalpinum*, *Aconitum columbianum*, *Mimulus guttatus*, *Cardamine infausta*, and sometimes willows.

There is usually a transition belt separating this meadow from the spruce forest. It is dominated by *Artemisia cana*, which is accompanied by *Dasiophora fruticosa*, *Agrostis hiemalis*, *Dugaldia hoopesii*, and *Eriogonum subalpinum*. The mesophytic meadows on the tops of the plateaus which adjoin the dry alpine scrub instead of the spruce forest lack the *Artemisia cana* border, which would seem to indicate that this sagebrush association is an outpost from the forest formation, rather than a successor to the meadow.

Certain alpine species which are uncommon below 10,000 feet are found in the higher rocky meadows. *Oxyria digyna*, *Sibbaldia procumbens*, *Primula parryi*, *Synthyris laciniata*, *Polemonium confertum*, *Filix fragilis*, and *Allium cernuum* are all found in moist crevices on high shaded cliffs.

10. Alpine scrub climax

On the summits of the High Plateaus, the subalpine forests often alternate with the dry alpine climax which occupies places where the soil is thin and exposure to the wind is great. The reduced size of the plants is evidence of the extreme exposure and the short season. This association seems to terminate a xerarch succession, but there is some evidence that a wet meadow may be replaced by it.

Placodium cinnabarinum and other crustose lichens (*Acarospora*, *Caloplaca*, *Lecanora rubina*, *L. saxicola*, and *Candelariella vitellina*) gain foothold on the smaller rock fragments as well as on the exposed

bedrock. Some foliose forms such as *Parmelia conspersa* and *Gyrophora hyperborea* also occur. The mosses *Tortula muralis* and *Hypnum revolutum* are seen in crevices with *Selaginella watsoni*. Apparently loose on stony soil is *Parmelia molliuscula*, a pale green foliose form which is blown about by the wind. Typical xerophytic alpine herbs follow. These are characterized usually by relatively large underground structures topped by a rosette of minute, often fleshy or hairy leaves and a short slender scape bearing the relatively large flowers. They develop rapidly when once the snow is gone, and mature their fruits before the short growing season is over. The commonest of these on the plateau summits are *Oreobroma pygmaea*, a fleshy leaved relative of our eastern spring beauty, *Androsace subumbellata*, *A. carinata*, *Sedum stenopetalum*, *Polygonum watsonii*, *Thlaspi glaucum* (?), *T. coloradense* (?), and a new *Thlaspi*, *Erigeron compositus* and its varieties *incertus* and *trifidus*, *Arenaria sajanensis*, *Draba mongollonica*, *D. crassifolia*, *Cerastium beeringianum*, *Artemisia scopulorum*, *Achillea lanulosa alpicola*, *Senecio petrophilus*, and species of *Antennaria*. As the soil deepens the alpine grasses take possession. The dominant species is *Festuca ovina* and its variety *brachyphyllum*. *F. rubra*, *F. thurberi*, *Agropyron tenerum*, and a little black sedge are also common at this stage. Between the tufts of grasses *Pseudocymopterus montanus*, *Achillea*, and *Sibbaldia procumbens* are found. The short fescue, *Festuca ovina*, gives the tone to these summit landscapes, but counts made in a number of meter quadrats show that other species, such as *Oreobroma pygmaea*, *Androsace subumbellata*, *Achillea*, and *Pseudocymopterus montanus*, although minute and inconspicuous, are very numerous.

The shallow troughs which drain the ponds and lakes on the summits may begin with a wet meadow association. Along the water courses are sedges, but on the wet banks grows *Caltha rotundifolia* in fairly pure stands. *Polygonum bistortoides* (and sometimes its variety *linearifolia*) and *Pedicularis groenlandica* are next, then *Phleum alpinum*, *Deschampsia caespitosa*, *Stipa columbiana*, *Saxifraga rhomboidea*, a white and a new purple flowered *Thlaspi*, *Sieversia turbinata*, *Phacelia sericea*, *Polemonium confertum*, *Potentilla concinna*, *P. glaucophylla*, *Sibbaldia procumbens*, *Senecio petrophilus*, and *Pseudocymopterus montanus* come in on the mid-slopes; at the top of

the slopes are the grasses and their associates. Here the wet meadow seems to be a pioneer stage preceding the dry tundra.

Wherever lava blocks afford some protection from the wind, or on leeward slopes, taller, more mesophytic herbs come in. These include blue and yellow columbines, *Anemone globosa*, *Phacelia sericea*, *Carex festiva*, *Mertensia pratensis*, *Sieversia triflora*, *S. turbinata*, *S. ciliata*, *Potentilla diversifolia*, *Drymocallis fissa*, *Heuchera parvifolia*, *Polemonium confertum*, a white *Polemonium*, *Erigeron* spp., and taller grasses such as *Poa sandbergii*, *Blepharineuron tricholepis*, *Koeleria cristata*, and *Deschampsia caespitosa*.

These rocks and taller herbs sometimes afford enough protection to permit the alpine shrubs *Juniperus sibirica* and *Ribes lentum* to come in. These shrub mats are more common on the central part of Fish Lake Plateau where large lava blocks were scattered by the glacier than on the other plateaus where the rock fragments are mostly small. They probably introduce the subalpine forest islands which dot the landscape on the Aquarius Plateau, but on the smaller plateaus the forests so developed are seldom much more than mere clumps of stunted and gnarled timberline trees. Whether they can compete against the wind and eventually establish a real climax spruce forest is a question. The timberline here seems to be determined by wind and by depth of soil rather than by temperature. At present the summits of all these High Plateaus represent a line of tension between the subalpine forest climax and the alpine scrub climax. Whether the true climax here is the subalpine forest, which is often long delayed owing to the slow disintegration of the rock and to desiccation by the wind, or the xerophytic alpine scrub which occasionally may be preceded by a more mesophytic subclimax which will eventually give way to it, is yet unsettled. Possibly both associations are true climax formations here, but each in its own climatic zone; for the climate on a broad plateau like the Aquarius Plateau may differ considerably from that on an isolated peak at the same altitude. At any rate, on the broader plateaus such as Aquarius Plateau, the two associations run rather parallel while on small tables like Fish Lake Plateau and Thousand Lake Mt. and on peaks like Mt. Marvine, where the wind is stronger, the alpine scrub takes the lead.

Plant list

KEY TO SYMBOLS USED TO DENOTE ZONES:

I, southern desert zone; IA, northern desert zone; IB, alkali desert
II, southern semidesert zone; IIA, northern semidesert zone
III, basal (western yellow pine) zone
IV, montane forest zone
V, subalpine forest zone
VI, alpine meadow climax
VII, alpine scrub climax

KEY TO SYMBOLS USED TO DENOTE HABITATS:

X, xerophytic; XX, extremely xerophytic
XM, xero-mesophytic
M, mesophytic
HM, hydro-mesophytic
H, hydrophytic
R, ruderal

I. LICHENS

(identified by the late Dr. PLITT of Baltimore, Md.)

Acarospora bella (Nyl.) Jatta.....crustose on lava
A. spp.....crustose on lava
Caloplaca spp.....crustose on lava
Candelariella vitellina Mull. Ary.....crustose on lava
Gyrophora hyperborea (Hoffm.) Mudd.....foliose on rocky soil
Lecanora rubina (Vill.) Wain.....semifoliose on rock
L. saxicola (Poll.) Ach.....semifoliose on rock
L. spp. (aspicilia?).....semifoliose on rock
L. divaricata (L.) Ach.....fruticose on spruces
Parmelia conspersa Ach.....foliose on rock, soil
P. molliuscula Ach.....foliose on rock
Peltigera spp.....foliose on moist soil
Usnea spp.....fruticose on spruces

II. MOSSES

(identified by Dr. SEVILLE FLOWERS)

Amblystegium riparium var. fluitans L. & S..... VI HM
A. riparium var. longifolium (Schultz) B. & S..... VI HM
A. serpens B. & S..... V HM
Aulacomnium palustre Schwaegr..... IV, VI HM
Brachythecium idahense R. & C..... V M

Bryum caespiticium L. ..... VI HM
B. capillare L. ..... VI HM
B. schleicheri Schwaegr. (?) ..... V M
B. spp. ..... VI HM
Climacium americanum Brid. ..... VI HM
Cratoneuron filicinum (L.) Roth. ..... VI HM
Drepanocladus aduncus var. Kneiffii form intermedius (B. & S.) Moenken ..... VI HM
D. uncinatus (Hedw.) Warnst. ..... VI HM
Hypnum revolutum Nutt. ..... VII X
Mnium affine var. rugicum B. & S. ..... VI HM
M. serratum Schrad. ..... V M
Philonotis fontana (L.) Brid. ..... IV, V, VI HM
Pohlia cruda (L.) Lindb. ..... VI HM
P. nutans (Schreb.) Lindb. ..... V M
Polytrichum juniperinum Willd. ..... V M
Swartzia montana (Lank.) Lindb. ..... V HM
Tortula muralis (L.) Hedw. ..... VII XX

III. Liverworts

Conocephalum spp. ..... III, IV, V HM
Marchantia spp. ..... III, IV, V HM

IV. Vascular plants

A. Trees

Abies concolor Lindl. ..... IV M
A. lasiocarpa (Hook.) Nutt. ..... V M
Acer interius Britton ..... I, II HM
Alnus tenuifolia Nutt ..... IV HM
Amelanchier alnifolia Nutt. ..... III, IV XM
A. oreophila A. Nels. ..... IIA XM
A. utahensis Koehne ..... I X
Betula fontinalis Sarg. ..... II–IV HM
Celtis rugulosa Rydb. ..... I HM
Cercocarpus intricatus Wats. ..... II X
C. ledifolius Nutt. ..... IIA, III, IV XM
C. parvifolius Nutt. ..... IIA, III, IV XM

Fraxinus anomala Torr. ..... I HM
Juniperus scopulorum Sarg. ..... II, III XM
J. utahensis (Engelm.) Lemm. ..... II XM
Picea engelmannii (Parry) Engelm. ..... V M
P. pungens Engelm. ..... II, III, IV HM, XM
Pinus aristata Engelm. ..... III XM
P. edulis Engelm. ..... II XM
P. flexilis James ..... V XM
P. ponderosa Dougl. ..... III XM
Populus angustifolia James ..... II HM
P. aurea Tidest ..... IV M
P. fremontii S. Watson ..... I HM
P. trichocarpa T. & G. ..... II HM
Prunus melanocarpa (A. Nels.) Rydb. ..... II, III, IV M
Pseudotsuga mucronata (Raf.) Sudw. ..... IV, III, V M, XM
Quercus utahensis Rydb. ..... IIA, III XM
Salix lasiandra Nutt. ..... I HM

B. Shrubs

Arctostaphylos platyphylla (A. Gray) Kuntze ..... III XM
A. uva-ursi (L.) Spreng. ..... III, IV, V XM
Artemisia cana Pursh ..... V, VI M
A. filifolia Torr. ..... I X
A. frigida Willd. ..... III, IV X
A. tridentata Nutt. ..... IA to IV X
A. tripartita Rydb. (?) ..... V, VI HM
Atriplex canescens (Pursh) Nutt. ..... IB X
A. confertifolia (Torr. & Gray) S. Watson ..... IB X
A. spp. ..... IB X
Ceanothus fendleri Gray ..... III, IV X
C. velutinus Dougl. ..... IV XM
Chrysothamnus graveolens (Nutt.) Greene .... IIA to IV X
Clematis ligusticifolia Nutt. ..... I HM
Coleosanthus microphylla Gray ..... I X
Cornus stolonifera Michx. ..... II, III HM
Cowania stansburiana Torr. ..... I, II, III X
Dasiophora fruticosa (L.) Rydb. ..... VI M

Ephedra torreyana S. Watson ..... I X
E. viridis Coville ..... I X
Eriogonum corymbosum Benth. ..... I X
E. effusum Nutt. ..... IIA XM
E. microthecum Nutt. (?) ..... IIA XM
E. (near polifolium Benth.) ..... IIA XM
Fallugia paradoxa (Don) Endl. ..... I, II, III X
Holodiscus dumosus (Nutt.) Heller ..... IIA XM
Juniperus sibirica Burgsd. ..... III, IV, V, VII XM
Lepargyrea argentea (Pursh) Greene ..... IV M
L. canadensis (L.) Greene ..... IV, V M
L. rotundifolia (Parry) Greene ..... I to III X
Lonicera involucrata Banks ..... IV M
Odostemon fremontii (Torr.) Rydb. ..... I XM
O. repens (Lindl.) Cockerell ..... III, IV, V M
Opulaster malvaceus (Greene) Kuntze ..... IV, V M
Pachystima myrsinites (Pursh) Raf. ..... III, IV, V M
Philadelphus microphyllus Gray ..... I X
Phoradendron juniperinum Engelm. ... (parasite on juniper)
Purshia tridentata DC. ..... III, IV XM
Rhus trilobata Nutt. (?) ..... I HM
R. utahensis L. N. Goodding ..... I X
Ribes cereum Dougl. ..... IV, V XM
R. inebrians Lindl. ..... III, IV XM
R. lentum (Jones) Coville & Rose ..... V, VII XM
R. leptanthum Gray ..... III XM
R. niveum Lindl. ..... V M
R. roezli Regel ..... V M
R. viscosissimum Pursh (?) ..... IV, V M
Rosa fendleri Crépin (?) ..... III, IV XM
R. manca Greene (?) ..... IV M
R. puberulenta Rydb. ..... I HM
R. pyrifera Rydb. (?) ..... IV M
R. woodsii Lindl. ..... V HM
Rubus hispidus L. ..... IV M
R. strigosus Michx. ..... IV, V, VII XM
Salix bebbiana Sarg. ..... V HM

S. exigua Nutt. ..... IV HM
S. lutea Nutt. (?) ..... IV HM
S. pseudomyrsinites var. aequalis Anderrs. (?) ..... V HM
S. subcoerulea Piper ..... IV HM
Sambucus melanocarpa Gray ..... III, IV XM
S. microbotrys Rydb. ..... IV, V M
Sarcobatus vermiculatus (Hook.) Torr. ..... IC X
Sericotheca microphylla Rydb. ..... IIA XM
Symphoricarpos longiflorus Gray ..... IV X
S. oreophilus Gray ..... IV XM to M
S. racemosus Michx. ..... III, IV XM
Tetradymia canescens DC. var. inermis Gray
IIA, III, IV X
Toxicodendron rydbergii Small ..... I XM
Yucca harrimaniae Trel. ..... I, II X
Y. spp. ..... I, II X

C. Herbs

Abronia elliptica A. Nels. ..... I, II X
A. spp. ..... III XM
Achillea lanulosa var. alpicola Rydb. ..... V, VI M
A. millefolium L. ..... III to V R
Aconitum columbianum Nutt. ..... IV, V HM
Agastache urticifolia (Benth.) Rydb. ..... V M
Agoseris purpurea (Gray) Greene ..... IV XM
Agropyron spicatum (Pursh) Scribn. & Wheeler ..... III XM
A. tenerum Vasey ..... IV HM
Agrostis hiemalis (Wolf) B.S.P. ..... III, IV HM
Allionia spp. ..... I X
Allium brandegii Hook. ..... III, IV HM
A. brevistylum Wats. ..... V HM
A. cernuum Roth. ..... VII HM
Allocarya scopulorum Greene ..... IV M
Alopecurus aequalis Sobol. ..... IV HM
A. geniculatus L. ..... IV HM
Ambrosia psilostachya DC. ..... I X
Andropogon scoparius Michx. ..... I X

Androsace carinata Torr. (?)	VII	X
A. diffusa Small	IV, V	M
A. subumbellata (A. Nels.) Small	VII	X
Anemone globosa Nutt	VI	HM
Antennaria aprica Greene	III	X
A. dimorpha (Nutt.) Torr. & Gray	VI	M
A. microphylla Rydb.	IV	HM
A. parvifolium Nutt	VI	HM
Apocynum androsaemifolium L.	IV	M
Aplopappus spp.	I	X
Aquilegia coerulea James	IV, V, VI	M
A. coerulea albiflora Gray	IV, V, VI	M
A. flavescens S. Wats.	VII	XM
A. pinetorum Tidest. (?)	IV	M
Arabis drummondii Gray	IV	M
Arenaria aculeata Wats.	I	X
A. sajanensis Willd.	VII	X
Argemone hispida Gray	I	R
Aristida fendleriana Steud.	IV	X
Arnica cordifolia Hook.	V	M
A. ocreata A. Nels.	V	HM
Artemisia scopulorum Gray	VII	X
A. wrightii Gray	II	X
Asclepias cryptoceras Wats.	I	XM
A. incarnata L.	I	XM
A. labriformis M. E. Jones	I	X
Aster adscandens Lindl. (?)	IV	HM
A. glaucodes Blake	IV	HM
Astragalus diversifolius Gray	II	XM
A. thompsonae S. Wats.	I, II	X
Atriplex spp.	IB	X
Batrachium grayanum (Freyn.) Rydb.	IV	H
B. trichophyllum (Chaix.) Barsch.	IV, V	H
Blepharineuron tricholepis (Torr.) Nash	VII	X
Bouteloua gracilis (H.B.K.) Lag.	I to III	X
B. hirsuta Lag.	IV	X
Bromus ciliatus L.	IV	HM

B. inermis Leyss. ..... III R
Calochortus nuttallii T. & G. ..... II, III XM
Caltha rotundifolia Greene ..... VI HM
Campanula parryi Gray ..... IV HM
C. uniflora L. (?) ..... VII X
Cardamine cordifolia Gray ..... V HM
C. infausta Greene ..... III to VI HM
Carex ebenea Rydb. ..... VI HM
C. engelmannii Bailey ..... VI HM
C. festiva Dewey ..... VII X
C. lanuginosa Michx. ..... IV HM
C. multinoda Bailey ..... IV HM
C. siccata Dewey ..... V, VI HM
C. variabilis Bailey ..... VI HM
C. spp. ..... VI HM
Castilleja arcuata Rydb. ..... V M
C. exilis A. Nels. ..... IV HM
C. flava A. Nels. ..... IV X
C. lauta A. Nels. ..... V M
C. linariaefolia Benth. ..... V M
C. linoides Gray (?) ..... IIA X
C. miniata Dougl. (?) ..... V M
C. parvula Rydb. (?) ..... IV X
C. rhexifolia Rydb. (?) ..... IV, V HM
Catabrosa aquatica (L.) Beauv. ..... IV, V H
Cenchrus pauciflorus Benth. ..... I HM
Cerastium beeringianum C. & S. ..... VII X
Chaenactis alpina (Gray) Jones (?) ..... VII X
C. douglasii H. & A. ..... IIA, III, IV X
Chamaesyce fendleri (T. & G.) Small ..... I, II X
Chamaenerion angustifolium (L.) Scop. ..... IV HM
Chrysopsis spp. ..... I HM
Chrysothamnus spp. ..... III, IV X
Chylismia spp. ..... I X
Clematis pseudoalpina (Kuntze) A. Nels. ..... III XM
Clementsia rhodantha (Gray) Rose ..... VI HM
Collomia linearis Nutt. ..... IV M

Comandra pallida A. DC. ..... IIA, III, IV XM
Corallorrhiza maculata Raf. parasite on roots ..... III XM
Crepis acuminata Nutt. ..... IV M
Datura meteloides DC. ..... I XM
Delphinium cockerellii A. Nels. ..... V M
D. subalpinum (Gray) A. Nels. ..... IV, V HM
Deschampsia caespitosa (L.) Beauv. ..... VI HM
Dodecatheon pauciflorum (Durand) Greene ..... VI HM
Draba aurea Vahl ..... V X
D. crassifolia Graham (?) ..... VII M
D. mongollonica Greene ..... VII X
D. spectabilis Greene (?) ..... VII X
Drymocallis fissa (Nutt.) Rydb. ..... VI M
D. spp. ..... VI M
Dugaldia hoopesii (Gray) Rydb. ..... VI M
Echinocactus xeranthemoides Coulter ..... I X
E. spp. ..... I X
Echinocereus fendleri (Engelm.) Rumpl. (?) ..... I X
E. goniocanthus (Engelm.) Lem. (?) ..... I X
E. mojavensis (Engelm.) Rumpl. ..... I X
E. triglochidiatus (in Britton & Rose) ..... I X
Eleocharis acicularis (L.) Roem. & Schultz ..... IV, V H
E. palustris (L.) Roem. & Schultz ..... IV, V H
Epilobium adenocaulon Hausskn. ..... IV, V HM
E. hornemannii Reichert (?) ..... V, VII HM
E. ovatifolium Rydb. ..... IV H
Equisetum arvense L. ..... III, IV, V HM
E. hiemale L. ..... III, IV H
Erigeron argentatus Gray ..... I, II X
E. bloomeri Gray ..... IV X
E. compositus Pursh ..... V, VII X
E. compositus discoideus Gray or incertus A. Nels. ... VII X
E. compositus trifidus Gray ..... VII X
E. eatonii Gray (?) ..... IV M
E. flagellaris Gray ..... IV X
E. formosissimum Greene ..... VI M
E. leiomerus Gray ..... I, II X

E. macranthus Nutt. ..... VI M
E. salsuginosus Gray ..... IV HM
E. superbus Greene ..... VI HM
E. ursinus D. C. Eaton (?) ..... IV, V M
E. vetensis Rydb. ..... III XM
Eriogonum alatum Torr. ..... III XM
E. inflatum Torr. ..... I X
E. jamesii Benth. ..... IV X
E. ovalifolium Nutt. ..... I, II X
E. piperi Greene ..... VI M
E. racemosum Nutt. ..... IIA, III, IV X
E. stellatum Benth. ..... III, IV X
E. subalpinum Greene ..... VI M
E. umbellatum Torr. ..... III, IV XM
E. spp. ..... other species in all zones
Euphorbia lurida Engelm. or
Tithymalus luridus (Engelm.) Woot. & Standl. ..... III XM
Eurotia lanata (Pursh) Moq. ..... IB X
Festuca ovina L. ..... V, VII X, XM
F. ovina brachyphylla Schult. ..... V, VII X
F. idahoensis Elmer ..... IV X
F. thurberi Vasey ..... V, VII XM
Filix fragilis (L.) Underw. ..... IV to VII XM
Fragaria bracteata Heller ..... V HM
F. glauca (S. Wats.) Rydb. ..... V HM
Franseria acanthicarpa (Hook.) Coville ..... I X
Frasera speciosa Griseb. ..... IV, V HM
Galium boreale L. ..... IV HM
Gayophytum ramosissimum T. & G. ..... IV X
Gentiana affinis Griseb. ..... V HM
G. calycosa Griseb. (?) ..... VI HM
G. elegans A. Nels. ..... IV HM
G. heterosepala Engelm. ..... VI HM
G. plebeja Cham. ..... VI HM
Geranium carolinianum L. ..... IV XM
G. richardsonii F. & M. ..... IV, V M
G. viscosissimum Fisch. & Mey. ..... IV to VII M

Geum macrophyllum Willd. ..... IV HM
G. strictum Ait. ..... IV HM
Gilia aggregata (Pursh) Spreng. ..... III, IV X
G. dixonae A. Nels. 1934 ..... I X
G. pulchella Dougl. ..... IV X
Glyceria striata Hitch. ..... IV HM
Gnaphalium uliginosum L. ..... VI HM
Gutierrezia filifolia Greene ..... III X
G. spp. ..... III, IV X
Habenaria elegans (Lindl.) Boland ..... IV HM
H. unalaskensis (Spreng.) Wats. ..... IV HM
Halerpestes cymbalaria (Pursh) Greene ..... I to V HM
Heuchera parvifolia Nutt. ..... V M
Hilaria jamesii (Torr.) Benth. ..... I, II X
Hippuris vulgaris L. ..... IV, V H
Hordeum nodosum L. ..... IV HM
Hymenoxys helenioides Rydb. ..... V, VI HM
H. odorata (DC.) Britt. ..... III X
H. richardsonii (Hook.) Ckll. ..... V R
Hypericum formosum H.B.K. ..... IV HM
H. scouleri Hook. ..... IV HM
Iris missouriensis Nutt. ..... IV HM
Iva axillaris Pursh ..... I X
Jonesiella asclepiadoides Rydb. ..... I X
Juncus balticus Willd. ..... III to VI H
J. filiformis L. ..... IV, V HM
J. saximontanus A. Nels. ..... IV, V HM
Kelloggia galioides Torr. ..... IV M
Koeleria cristata L. ..... VI HM
Lappula floribunda (Lehm.) Greene ..... IV XM
L. occidentalis (S. Wats.) Greene ..... IV XM
Lathyrus leucanthus Rydb. ..... IV, V M
L. spp. ..... V M
Lavauxia flava A. Nels. ..... IV, V M
L. howardii M. E. Jones ..... V HM
Leontodon scopulorum Rydb. ..... V HM
Lepidium jonesii Rydb. ..... I XM

Lesquerella alpina (Nutt.) Wats. ..... III X
Ligusticum porteri Coulter & Rose ..... IV M
Linum lewisii Pursh ..... IV M
Lithospermum multiflorum Torr. Wats. ..... IV M
Lotus wrightii (Gray) Greene ..... III, IV XM
Lupinus alpestris A. Nels. ..... III, IV, V XM
L. argenteus Nutt. ..... IIA X
L. lepidus Dougl. ..... III XM
L. parviflorus Nutt. ..... V HM
L. pseudoparviflorus Rydb. (?) ..... V HM
L. spp. ..... II to VII
Lygodesmia exigua Gray ..... IIA X
L. juncea (Pursh) D. Don ..... II X
L. spinosa Nutt. or Pleiacanthus spinosus (Nutt.) Rydb. ..... II to IV X
Melilotus alba Desv. ..... I R, HM
M. officinalis (L.) Lam. ..... I R, HM
Mertensia arizonica Greene (?) ..... IV HM
M. pratensis Heller ..... IV, V HM
Microseris spp. ..... IV X
Mimulus guttatus DC. ..... IV, V H
Mitella spp. ..... V M
Monardella odoratissima Greene ..... V XM
Moneses uniflora (L.) Gray ..... V HM
Montia chamissoi (Ledeb.) Tidest. ..... V HM
Muhlenbergia asperifolia Nees. & Mey. ..... IB HM
Myosurus aristatus Benth. ..... IV, V HM
Myriophyllum spicatum L. ..... IV H
Opuntia fragilis (Nutt.) Haw. ..... II, III, IV X
O. polyacantha Haw. (?) ..... I, II X
O. rhodantha Schum. ..... II X
O. rutila Nutt. ..... I, II X
O. xanthostemma Schum. ..... II X
O. spp. ..... I, II X
Oreobroma pygmaeum (Gray) Howell ..... VII X
Oreocarya flava A. Nels. (?) ..... I X
O. setosissima (Gray) Greene (?) ..... IV XM

O. (near suffruticosa (Torr.) Greene) ..... III X
Oreoxis alpina (Gray) C. & R. ..... V M
Orobanche ludoviciana Nutt. parasitic on Chrysothamnus ..... II X
Orthocarpus luteus Nutt. ..... V M
Oryzopsis hymenoides (Roem. & Schult.) Ricker ..... II X
Osmorrhiza obtusa (Coulter & Rose) Fernald ..... IV, V M
Oxyria digyna (L.) Hill ..... VII XM
Oxytropis deflexa (Pall.) DC. (?) ..... V XM
Pachylophus macroglottis Rydb. ..... IV X
Parnassia parvifolia DC. ..... V HM
Pedicularis bracteosa Benth. ..... VI XM
P. groenlandica Retz. ..... VI HM
P. groenlandica surrecta (Benth.) Greene ..... VI HM
P. parryi Gray ..... VI M
Pediocactus simpsonii (Engelm.) Britton & Rose. III, IV X
Pentstemon arenicola A. Nels. ..... III X
P. caespitosus Nutt. ..... II XM
P. eatonii undosus M.E. Jones ..... II X
P. ophianthus Pennell ..... II XM
P. pachyphyllus Gray ..... III XM
P. procerus Dougl. ..... VI M
P. procerus pulverens Pennell ..... VI M
P. strictus Benth. ..... IV X
P. subglaber Rydb. ..... V M
P. torreyi Gray (?) ..... III XM
P. whippleanus Gray ..... V HM
P. spp. ..... I to VII X to M
Phacelia corrugata A. Nels. ..... I XM
P. idahoensis Henders. ..... IV M
P. sericea Gray ..... IV, V, VI, VII M, XM
P. spp. ..... IIA X
Phleum alpinum L. ..... VI HM
Phlox austromontana Coville ..... III XM
P. canescens Torr. & Gray ..... I XM
P. cortezana A. Nels. ..... V M
P. stansburyi (Torr.) Heller ..... III, IV XM

Physaria didymocarpa Gray..... III XM
Poa ampla Merr..... VI M
P. fendleriana (Steud.) Vasey..... IV, V M
P. longiligula Scribn. & Wms..... V M
P. pratensis L..... IV, V M
P. sandbergii Vasey..... VI, VII M
Polemonium albiflorum Eastw. (?)..... VII X
P. confertum Gray..... VII X
P. foliosissimum Gray (?)..... IV HM
P. pulcherrimum Hook..... VII X
P. viscosum Nutt. (?)..... VII X
Polygonum amphibium L..... IV, V H
P. bistortoides Pursh..... VI, VII HM
P. bistortoides linearifolia S. Wats..... VI, VII HM
P. watsonii Small..... VII X
Potamogeton americanus Schlecht. & Cham..... IV, V H
P. spp..... IV, V H
Potentilla concinna Rich..... VII X
P. (near concinnaeformis Rydb.)..... VII X
P. dichroa Rydb..... III XM
P. diversifolia Lehm..... IV XM
P. effusa Dougl., Lehm..... V HM
P. fastigiata Nutt..... IV HM
P. glaucophylla Lehm..... IV, HM to VII XM
P. gracilis Dougl..... IV M
P. hippiana Lehm. (?)..... IV HM
P. intermittens Rydb. (?)..... IV HM
P. monspeliensis L..... V HM
P. nuttallii Lehm..... IV HM
P. pennsylvanica L..... III HM
P. propinqua Rydb..... IV HM
P. spp..... IV–VII
Primula parryi Gray..... V, VII HM
Pseudocymopterus montanus (Gray) Coulter & Rose
III, IV, V XM
P. spp..... VII X
Pyrola asarifolia Michx..... V HM

P. chlorantha Sw. ..... V HM
P. secunda L. ..... III, IV HM
Quamoclidion froebelii (Behr.) Standl. ..... I XM
Ranunculus alpeophilus A. Nels. ..... V HM
R. calthaeflorus Greene ..... VI HM
R. cardiophyllus Hook. ..... VI HM
R. cymbalaria Pursh or Halerpestes cymbalaria (Pursh) Greene ..... I to V HM
R. escholtzii Schlect. ..... IV HM
R. inamoenus Greene ..... V HM
R. montanensis Rydb. ..... IV HM
R. sceleratus L. ..... III HM
Roripa curvipes Greene ..... V HM
R. sinuata (Nutt.) A.S.H. ..... IV HM
Rumex mexicanus Meisn. ..... IV HM
Sagina saginoides (L.) Britton ..... VII HM
Salsola pestifer A. Nels. ..... I X
Saxifraga arguta D. Don ..... V M
S. integrifolia sierrae Coville ..... V M
S. rhomboidea Greene ..... V HM
Sedum stenopetalum Pursh ..... VII X
S. spp. ..... VII X
Selaginella mutica D. C. Eaton ..... II X
S. watsoni Underw. ..... VII X
Senecio atratus Greene ..... V M
S. crocatus Rydb. ..... IV HM
S. cymbalarioides Nutt. ..... VI M
S. multilobatus Torr. & Gray ..... IV X
S. mutabilis Greene ..... IV HM
S. petrophilus Greene ..... VII X
S. purshiana Nutt. (?) ..... VII X
S. triangularis Hook. ..... V X
S. spp. ..... several other species in zones III–VII
Sibbaldia procumbens L. ..... VII XM
Sidalcea candida Gray ..... IV HM
Sieversia ciliata G. Don ..... VI, VII M
S. triflora (Pursh) R. Br. ..... VI, VII XM

S. turbinata (Rydb.) Greene ..... VII X
Silene antirrhinus L. ..... V M
Sisyrinchium angustifolium Nutt. ..... IV HM
Sitanion hystrix (Nutt.) J. C. Smith ..... III X
Sium cicutaefolium Gmelin ..... V HM
Smilacina stellata (L.) Desf. ..... III HM
Solidago bigelovii Gray ..... IIA X
Sophia filipes (Gray) Heller ..... VII X
S. hartwegiana (Fourn.) Greene ..... IV HM
Sphaeralcea grossulariaefolia (H. & A.) Rydb. ..... I, II X
Stanleya integrifolia James ..... I HM
Stipa columbiana Macoun ..... IV to VII X
S. comata intermedia Scribn. ..... IV X
S. speciosa Trin. & Rupr. ..... I, II X
Synthyris laciniata (Gray) Rydb. ..... V HM
Taraxacum officinale Web. ..... II to VII R
Taraxia breviflora (T. & G.) Nutt ..... IV, V HM
T. subacaulis (Pursh) Rydb. (?) ..... V M
Thalesia fasciculata (Nutt.) Britton, parasite on Artemisia ..... IIA X
Thalictrum fendleri Engelm. ..... IV HM
T. megacarpum Torr. ..... V M
T. occidentale Gray ..... V M
Thermopsis montana Nutt. ..... IV, V, VI M
Thlaspi coloradense Rydb. (?) ..... VII X
T. glaucum A. Nels. (?) ..... VII X
T. spp.—a new purple-flowered species ..... VII X
Tithymalus luridus (Engelm.) Woot. & Small ..... III XM
Townsendia arizonica Gray ..... I X
T. spp. ..... I X
Trifolium rydbergii Greene ..... VI M
T. repens Wheeler ..... IV HM, R
Trisetum subspicatum (L.) Beauv. ..... IV M
Urtica breweri S. Wats. ..... IV HM
Valeriana ceratophylla (Hook.) Piper ..... IV HM
Veratrum speciosa Rydb. ..... V HM
Veronica alpina L. ..... IV HM

V. americana Schwein..... III HM
V. serpyllifolia L..... IV HM
V. wormskjoldii Roem. & Schult..... V HM
Vicia americana Michx..... IV M
Viola macloskeyi Lloyd or V. orbiculata Geyer..... V HM
V. nephrophylla Greene..... III HM
Woodsia oregana D. C. Eaton..... III XM
Wyethia scabra Hook..... I X
Zygadenus elegans Pursh..... V, VI HM

Summary

1. An ecological survey was made of the vegetation of the region between the 111th and the 112th meridians west of Greenwich and the 38th and 39th parallels north of the equator. It lies northeast of Bryce Canyon and northwest of the Henry Mts.

2. The vegetation in this region has been little disturbed except to a small extent by lumbering and to a somewhat greater extent by grazing.

3. Studies were made from the rock desert regions in the eastern part of this quadrat up to the summits of the plateaus included within its borders, namely, Aquarius, Thousand Lake, Fish Lake, and Awapa of the High Plateaus and the monoclines known as the Water Pocket Flexure and Miner's Mountain.

4. The High Plateaus are glaciated, lava-capped uplifts due to faulting, whose summits are 11,000 feet and more in altitude. The Water Pocket Flexure and Miner's Mountain are monoclines of sedimentary strata which reach to 8250 feet in altitude and are dissected by numerous gorges.

5. The climate varies from desert in the lowlands at 5000 feet where the annual precipitation is 5 to 6 inches to alpine on the summits.

6. There is a wide variety of soils, both residual and transported.

7. The flora includes representative formations from southern desert to alpine scrub, the lowlands lying on the outskirts of Lower Sonoran territory and the summits on the tension line between the subalpine forest and the arctic-alpine desert.

8. The southern flora extends up the tributaries of the Colorado

system, but corresponding levels on the Great Basin slopes of the tables lack these species.

9. The timber line is determined by wind rather than by temperature, so the broader the summit the better the development of forest. Small tables and isolated peaks are occupied mostly by alpine scrub; larger tables, by parklike arrangements of scrub and forest.

10. As far as possible the various successions to the climax in each climatic zone were worked out.

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CHICAGO, ILLINOIS

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LIPASE PRODUCTION BY PENICILLIUM OXALICUM AND ASPERGILLUS FLAVUS[1]

DAVID KIRSH

Introduction

The fact is well established that the function of lipases, the group of enzymes hydrolyzing fats and oils, is as important in animal, plant, and microbial metabolism as are the other two large groups of enzymes, the proteases and carbohydrases. Among the different lipases, those produced by microorganisms, particularly the fungi, have been least studied and are therefore least understood, in spite of the fact that this subject had its beginning in 1897, when GERARD (5) reported the elaboration of this enzyme by a green species of *Penicillium*. Among the enzyme systems of fungi the carbohydrases and proteases have received most attention. Papers dealing with the enzymes acting on fats have been of a desultory nature, and, with few exceptions, concern themselves merely with the statement that certain fungi have been shown to possess the property of producing lipase (13).

Lipase production by *Aspergillus niger* has been found (4) to reach a maximum long after sporulation of the fungus, although TAKAMINE (12) and OSHIMA and CHURCH (8) report that enzyme production by *A. oryzae* on a solid medium is greatest just at the time of complete sporulation. The presence of coconut oil in the culture medium stimulates lipase production by various fungi (2), while the lipase content of koji (steamed rice infected with *A. oryzae*) was observed to be almost proportional to the fat content of the raw materials (6). *A. niger* (11) was also found to produce more lipase on a fat-containing medium than on a cane sugar or glycerol medium.

In the present investigation, solid media were employed in order to obtain a dry fungus lipase preparation similar to that of taka-

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diastase from *Aspergillus oryzae*, in quantities large enough to permit a detailed study of the properties and mode of action of the enzyme.

Experimentation

A series of fungi capable of growing upon fatty subtances were isolated from various sources, namely, soil, sewage, forest organic matter, decomposing Brazil nuts, etc., employing the customary enrichment culture method (except in the case of the Brazil nuts, in which the organisms were isolated directly) and plating out upon Rahn's medium (3) containing olive oil. Water was added to the source material if necessary to create optimum moisture conditions and sufficient $(NH_4)_2HPO_4$ to give a final concentration of 0.25 per cent. Rahn's medium was adjusted to neutrality and 0.1 per cent brom thymol blue added. The enrichment culture material was plated out and incubated at 28° C.; those colonies which grew rapidly and produced sufficient acid from the olive oil to change the color of the indicator from blue to yellow were selected for preliminary study. Various species of *Aspergillus*, *Penicillium*, *Oidium*, *Mucor*, *Trichoderma*, and *Monilia*, as well as several unidentified bacteria and actinomyces, were thus isolated.

The lipolytic activity of the fungi was determined in the following manner: 30 gm. of a substrate consisting of one part bran, one part wood flour, three parts of a 1 per cent $(NH_4)_2HPO_4$ solution, and sufficient olive oil to give a final concentration of 5 per cent, were placed in pint Mason jars or some similar glass containers, plugged with cotton, sterilized in intermittent flowing steam, and inoculated with 3 cc. of a water suspension of spores. The spores were obtained from a dried stock culture prepared in a similar manner. The seed was used only after several generations of the fungus originally grown on an agar medium had been cultured on the solid medium, in order to allow the organism to adapt itself to the substrate.

Since most workers agree that the quantity of extracellular enzyme is greatest just after complete fructification, the cultures were analyzed after three to six days' incubation, during which time sporulation was complete. The cultures were ground with sand and 80 cc. of distilled water, allowed to stand for one hour, and then

filtered into cheesecloth. Most of the water was removed in a squeezing device similar to a potato ricer, the rest of the water being expressed in a small fruit press. The extract was centrifuged for 25–30 minutes, and an aliquot representing one-fourth (made up to 25 cc.) placed in a 250 cc. Erlenmeyer flask together with 1 cc. of 0.1 N H_2SO_4 (to adjust to approximately pH 5.0), 5 cc. of a pH 5.0 phosphate buffer, and 5 cc. of a 50 per cent olive oil emulsion containing 2.5 per cent gum arabic (**9**). After the addition of 1 cc. of toluene, the flasks were stoppered, shaken, and incubated at 40° C. for 20 hours. The flasks, of which there were always two identical series: an unboiled series designated in the tables under the heading Enzyme, and a boiled control series in which the extract was boiled for five minutes to destroy the enzyme, were titrated with 0.1 N NaOH against phenolphthalein after the addition of 75 cc. of 95 per cent alcohol and 25 cc. of ethyl ether. Lipolytic activity is arbitrarily expressed for comparative purposes as the number of cc. of 0.1 N acid split from the olive oil by the extract obtained as described. The details concerning the factors influencing the activity of the enzyme will be found elsewhere (**7**).

The two most active cultures of the many tested were found to be a form of *Penicillium oxalicum* (isolated from a deciduous forest soil using cod-liver oil in the enrichment culture), and a strain of *Aspergillus flavus*[2] (isolated from decomposing Brazil nuts); these organisms were employed in most of the subsequent studies.

LIPASE PRODUCTION ON A SOY BEAN MEDIUM.—In many of the early experiments employing the bran-wood flour-olive oil medium, no more than about 7 cc. of 0.1 N acid was split off from the olive oil by the enzyme, this figure representing about 9 per cent hydrolysis (5 cc. of the olive oil emulsion theoretically yields 78 cc. 0.1 N acid on complete hydrolysis when the saponification value of the oil is 190). In order to determine whether lipase production would be enhanced if the organisms were grown on some natural oil-containing material, a medium was made up of 10 gm. of ground soy beans

[2] The writer is indebted to Dr. CHARLES THOM of the U.S. Department of Agriculture for the identification of these two species. Concerning the two organisms Dr. THOM writes, "The *Aspergillus flavus* is of the usual general type. The *Penicillium* has the spore-producing mechanism of *Penicillium oxalicum*, but a very woolly growing surface entirely different in general appearance from the form we commonly find in the soil."

(Harbinsoy variety containing 20.3 per cent oil), 10 gm. of bran, and 20 cc. of water. The fungi grew rapidly and luxuriantly, the extracts hydrolyzing the olive oil to the extent of 35 per cent in one experiment and 50 per cent in another. The results in table I, as in all subsequent tables, are reported as cc. of 0.1 N acid, and indicate the amount of cleavage brought about after 6 and 20 hours of incubation of the fungus extracts and olive oil.

TABLE I

LIPOLYTIC ACTIVITY OF EXTRACTS OF FUNGI GROWN ON SOY BEAN MEDIUM (CC. OF 0.1 N ACID)

	Hours					
	Penicillium oxalicum			Aspergillus flavus		
	0	6	20	0	6	20
Enzyme	.....	57.3	74.8	.....	66.0	77.8
Boiled control	.....	35.5	35.0	.....	51.3	51.0
Activity	.....	21.8	39.8	.....	14.7	26.8
Unboiled control	35.3	38.8	40.0	50.0	54.5	56.5

The unboiled control differed from the boiled control in that the enzyme was not destroyed by heat in the former and did not have any oil emulsion added. The former control value is higher than the latter since it allows for the action of the enzyme during the incubation period on oil extracted from the medium. For all practical purposes, however, the boiled control value is used in calculating enzymic activity, since this figure gives a measure of the total enzyme present in the extract which can be obtained in a dry powder form. For comparative purposes, the unboiled control values are occasionally given.

By following the changes in amino nitrogen content in the unboiled controls, it was definitely shown that the increase in titratable acidity in the enzyme flasks was not due to any protease action. The slight increase in amino nitrogen over a period of 20 hours' incubation corresponded to only about 2–3 cc. 0.1 N acid, a value which is negligible when one considers the acidity resulting from the action of the enzyme on the oil.

INFLUENCE OF OIL CONTENT OF MEDIUM.—To 30 gm. portions of the bran-wood flour medium was added sufficient olive oil to give concentrations ranging from 0 up to 30 per cent of the final weight. At a concentration of 20 per cent oil the physical condition of the medium was only fair, and at 30 per cent it was poor, being rather soggy owing to the large excess of oil. After four days' incubation the medium containing no oil had only a thin growth which failed to bind the substrate together. With increasing quantities of oil, the growth of the fungus was more abundant and the spores darker in

TABLE II

INFLUENCE OF CONCENTRATION OF OIL IN GROWTH SUBSTRATE ON LIPASE PRODUCTION BY FUNGI (CC. OF 0.1 N ACID)

PERCENTAGE OIL ADDED TO MEDIUM	PENICILLIUM OXALICUM			ASPERGILLUS FLAVUS		
	ENZYME	CONTROL	ACTIVITY	ENZYME	CONTROL	ACTIVITY
0	35.8	24.0	11.8	30.4	22.8	7.6
2½	52.0	25.2	26.8	28.0	22.5	5.5
5	51.0	24.5	26.5	27.3	23.0	4.3
10	32.0	24.0	8.0	30.1	26.6	3.5
15	30.9	24.6	6.3	39.0	34.8	4.2
20	31.1	25.5	5.6	43.5	38.9	4.6
30	36.3	29.4	6.9	.....	.....	.....

color, a compact mat being produced in concentrations of 5 per cent and upward. The extracts from the 20 per cent oil medium contained a slight amount of free oil after centrifuging, while the 30 per cent oil medium extract contained about 4 cc. of free oil. Aliquot portions of the oil were removed with the extracts in setting up the enzyme flasks.

The results in table II point to the fact that a medium containing from 2.5 to 5 per cent oil is most favorable; lipase formation by *Penicillium oxalicum* is greatest on this medium. The results are not so definite in the case of *Aspergillus flavus*, owing to its low activity; nevertheless they point to the same conclusion. It was first believed that the richer medium resulted in a greater production of heat by the organisms, this heat having a destructive effect upon the enzyme. When the experiment was repeated with the cultures incubated in a water bath at 28° C. (the advantage of this manner of

incubation is pointed out in a later experiment), the same general results were obtained.

The same type of experiment was employed with the soy bean medium using several varieties of soy beans having different oil contents, and ether-extracted beans to which definite quantities of soy bean oil were added. The results did not bring out any significant differences. It is possible that when different varieties of soy beans are employed the nature of the nutrients and the nitrogen content play important rôles in the metabolism of the organisms concerned. On the other hand, the actual differences in oil content were not

TABLE III

EFFECT OF PERIOD OF INCUBATION ON LIPASE PRODUCTION (CC. OF 0.1 N ACID)

INCUBATION PERIOD OF CULTURES IN DAYS	PENICILLIUM OXALICUM			ASPERGILLUS FLAVUS		
	ENZYME	CONTROL	ACTIVITY	ENZYME	CONTROL	ACTIVITY
2	42.5	24.0	18.5	66.3	44.4	21.9
3	55.6	26.3	29.3	73.0	51.1	21.9
4	62.6	30.2	32.4	79.5	60.5	19.0
5	53.2	29.4	28.8	78.3	59.9	18.4
6	60.2	35.8	24.4	84.0	69.0	15.0
7	58.0	34.1	23.9	86.2	67.5	18.7
10	57.2	38.2	19.0	81.0	66.0	15.0
13	61.5	45.4	16.1	89.0	76.6	12.4
17	65.1	52.7	13.4	88.3	75.5	12.8
30	60.5	53.2	7.3	85.0	73.3	11.7

great, since the extent was only from zero to 2 gm., whereas it went up to 12.9 gm. in the olive oil series.

EFFECT OF PERIOD OF INCUBATION.—As shown in table III, the lipase content reaches a maximum in both cultures after three to four days' incubation; after this it decreases slowly, so that after 30 days there is still an appreciable amount in the cultures. Fructification began after two days, and appeared to be complete after four days. The drying of the cultures on incubation may have had a preserving effect on the enzyme and thus account for its slow disappearance in the older cultures. In some large scale experiments, where greater quantities of medium were employed, fructification was considerably delayed.

EFFECT OF TEMPERATURE OF INCUBATION.—A series of jars was inoculated with *Penicillium oxalicum* and incubated in water baths at various temperatures. The water in the baths was kept constantly agitated by a small stream of air bubbles, thus causing a continuous circulation about the culture jars. A duplicate series was placed in a hot air incubator at 28° C., as well as in water at this temperature. The cultures were analyzed after four days' incubation while an extra series was kept at the lower temperatures for a longer period to permit fructification of the fungus. All the cultures were held at 28° C. for about 28 hours to permit good mycelial development,

TABLE IV

EFFECT OF TEMPERATURE OF INCUBATION OF PENICILLIUM OXALICUM ON LIPASE PRODUCTION (CC. OF 0.1 N ACID)

INCUBATION TEMPERATURE (°C.)	INCUBATION 4 DAYS			INCUBATION 6 DAYS		
	ENZYME	CONTROL	ACTIVITY	ENZYME	CONTROL	ACTIVITY
13	30.2†	21.3	17.9	42.9§	23.4	19.5
18.5	55.5	29.3	26.2	50.8	31.5	19.3
24	69.5	35.0	34.5	71.6	33.4	38.2
28	75.5	32.1	43.4	.....	.....	.....
28 (air)*	59.5	27.7	31.8	.....	.....	.....
35	62.5‡	29.5	33.0	.....	.....	.....

* This set of cultures was kept in an electric hot air incubator; all the other sets were kept in water baths at the various temperatures.

† No sporulation in 4 days at this temperature.

‡ Poor sporulation.

§ This set of cultures was incubated for 8 days to allow sporulation.

after which time they were transferred to their respective water baths.

From table IV it appears that the greatest quantity of lipase is obtained when the fungus is incubated at 28° C. in a water bath. When kept at this temperature in air the yield is lower, owing to the heat generated by the growing organism. Air, a poor conducting medium, does not remove this heat as efficiently as does circulating water. In some cases the temperature of cultures placed in a 28° C. air incubator has been observed to go as high as 38° C., a temperature harmful to the enzyme. TAKAMINE (**12**) has reported temperatures as high as 42° C. in rooms where *Aspergillus oryzae* is originally

started at 30° C. for the manufacture of taka-diastase. In such cases the rooms are cooled to prevent destruction of the enzyme.

OPTIMUM CONCENTRATION OF SOY BEAN IN SUBSTRATE.—Jars of media were prepared in which the soy bean content was varied from 0 to 12.5 gm. with 2.5 gm. variation in each series. Correspondingly, the amounts of bran were varied so that the total quantity of solid material was 20 gm. When 12.5 gm. of soy bean was mixed with 7.5 gm. of bran, the resultant medium, after the addition of water, had poor physical condition, being soggy and pastelike. Two jars were also prepared containing 5 gm. of bran, 5 gm. of wood flour,

TABLE V

EFFECT ON LIPASE PRODUCTION OF VARYING RATIO OF SOY BEAN TO BRAN IN GROWTH MEDIUM OF PENICILLIUM OXALICUM (CC. OF 0.1 N ACID)

MEDIUM		ENZYME	CONTROL	ACTIVITY
BRAN (GM.)	SOY BEAN (GM.)			
20.0	0	49.8	30.7	19.1
17.5	2.5	64.3	32.6	31.7
15.0	5.0	66.9	31.7	35.7
12.5	7.5	72.1	32.1	40.0
10.0	10.0	67.9	32.0	35.9
7.5	12.5	69.9	35.7	34.2
5.0*	10.0	51.3	29.1	24.2

* Mixed with 5.0 gm. wood flour.

and 10 gm. of soy bean. The medium thus obtained was of a fine porous texture. The jars were inoculated with *Penicillium oxalicum*.

The results in table V indicate that a ratio of 7.5 gm. of soy bean to 12.5 gm. of bran yields the best medium for lipase production. When a still finer series was set up, employing quantities of soy beans ranging from 5 to 10 gm. at intervals of 1 gm., and correspondingly, quantities of bran ranging from 15 to 10 gm., no significant differences were obtained. In further experiments, therefore, the optimal ratio of ingredients was employed.

INFLUENCE OF VARIOUS PLANT MATERIALS IN SUBSTRATE ON LIPASE PRODUCTION BY PENICILLIUM OXALICUM.—A series of media containing ground cotton seeds, cotton seed meal, flax seeds, flax seed meal, soy beans, and soy bean meal, were inoculated with *P. oxali-*

cum. From the results in table VI, it appears that lipase production is greatest on the soy bean medium. The seeds and the meals (seeds from which most of the oil is removed by pressure) appear to serve equally well as a substrate for enzyme production except in the case of soy bean. Here the yield is twice as great on the bean as it is on the meal. In a repetition of this experiment employing the soy bean and the meal, the activity on the former was 26.3 cc. 0.1 N acid, and on the latter 21.4 cc. 0.1 N acid, thus conforming more to the results obtained with the other plant materials.

TABLE VI

INFLUENCE OF VARIOUS PLANT MATERIALS IN SUBSTRATE ON LIPASE PRODUCTION BY PENICILLIUM OXALICUM (CC. OF 0.1 N ACID)

PLANT MATERIAL IN SUBSTRATE	ENZYME	CONTROL	ACTIVITY
Cotton seeds	63.3	35.5	27.8
Cotton seed meal	69.3	41.4	27.9
Flax seed	61.5	37.0	24.5
Flax seed meal	58.6	35.5	23.1
Soy beans	74.0	39.7	34.3
Soy bean meal	74.4	57.0	17.4
Soy beans*	81.0	54.7	26.3
Soy bean meal*	81.0	59.6	21.4

* These results were obtained in a separate experiment.

PREPARATION OF A DRY FUNGUS LIPASE.—*Penicillium oxalicum* was inoculated into 1600 gm. of moist soy bean medium. After five days' incubation the cultures were ground and extracted twice with water, the extracts being removed in an hydraulic press at 3000 pounds pressure per square inch. The combined extracts after centrifuging were poured slowly into four volumes of 95 per cent alcohol which was being agitated thoroughly by an electric stirrer. The precipitate was subjected to two more treatments with three volumes of alcohol, and once to one volume of acetone. The enzyme was filtered off and dried for several hours in an oven by means of a current of air warmed to 40° C. From the activity of the extract and the precipitated enzyme, it was calculated that slightly more than 80 per cent of the lipase was recovered in the dry powder.

Table VIIA shows the activity of the dry enzyme together with that of a high lipase trypsin of pancreatic origin for comparative purposes. The activity of the preparation thus obtained was rather low, as was that of the extract of the culture. This may have been due to the fact that the culture was kept in an air incubator instead

TABLE VIIA

LIPOLYTIC ACTIVITY OF DRY ENZYME PREPARATIONS (CC. OF 0.1 N ACID)

LIPASE EMPLOYED	QUANTITY OF LIPASE (MG.)	pH	ENZYME	CONTROL	ACTIVITY
Penicillium oxalicum.....	125	5.0	18.4	13.0	5.4
	250	5.0	22.8	14.0	8.8
High lipase trypsin.....	125	5.0	26.6	13.0	13.6
		8.0	27.0	2.7	24.3
		8.9	42.6	35.2	7.4

TABLE VIIB

LIPOLYTIC ACTIVITY OF A DRY LIPASE OF PENICILLIUM OXALICUM (CC. OF 0.1 N ACID)

QUANTITY OF LIPASE (MG.)	ENZYME	CONTROL	ACTIVITY	HYDROLYSIS OF OLIVE OIL (%)*
125	28.3	13.1	15.2	19.49
250	37.4	15.0	22.4	28.72

* Not corrected for acidity resulting on incubating enzyme solution alone.

of in a water bath, and also that it was removed before good sporulation had set in.

A similar preparation was later obtained from some cultures grown in 40 gm. lots of medium which gave five times as much enzyme on a yield times activity basis as the previous experiment. The degree of hydrolysis brought about by this preparation was three times as great as that obtained with the previous one (table VIIB).

A large scale experiment was recently attempted whereby 9.5 kg. of medium containing 51 per cent added moisture was inoculated

and kept in shallow layers in a water bath. The experiment was not conducted under pure culture conditions, it being assumed that a heavy inoculum would enable the fungus to grow quickly and crowd out any contaminants. The experiment proceeded successfully, and 380 gm. of dry enzyme was obtained, but unfortunately its lipolytic activity was very low.

It is of interest to note here that after four days' incubation there was a loss in dry weight of the culture of 31.5 per cent.

Discussion

It is apparent from an examination of the data that the activity of the extracts obtained in different experiments showed considerable variation. Thus the activity of *Penicillium oxalicum* extracts ranged from as high as 42.0 cc. 0.1 N acid down to 9 cc. These variations may be attributed to many things: the different lots of seed employed, variation in the amount of seed used for inoculation, etc. For this reason only results within the same experiment should be compared.

Many other results were obtained in this study which do not require presentation in tabular form. Thus hot water extraction of the wood flour or bran used in the olive oil medium did not effect lipase production, although washed bran yielded a lighter colored extract.

With the bran-wood flour-olive oil medium, asparagine, peptone, or KNO_3 offered no advantage over $(NH_4)_2HPO_4$ in the case of either organism. The addition of the mineral elements present in Rahn's nutrient solution for fat-splitting organisms exerted no beneficial effect. On the soy bean medium no additional minerals or nitrogenous materials were required. When $(NH_4)_2HPO_4$ was added to this medium in concentrations greater than 2 per cent of the dry medium, a diminution in lipase production was observed.

Where higher concentrations of olive oil in the bran-wood flour medium resulted in a lower yield of lipase, it is possible that the excess of metabolic by-products may have inactivated the enzyme or destroyed it as suggested earlier by DELEANO (1).

Although a very active dry lipase was not obtained during the course of the work, the fungus preparation did contain eight and

a half times more lipase per unit of protease than did the commercial pancreatic high lipase trypsin. The method therefore holds promise of yielding a preparation of comparatively high lipase and low protease activity. It remains to be seen why the organism fails to produce as active an enzyme when grown in larger quantities as it does in 40 gm. lots of medium.

Summary

1. *Penicillium oxalicum* and *Aspergillus flavus* produce a water soluble enzyme capable of hydrolyzing olive oil when grown on a bran-wood flour-olive oil medium or a bran-soy bean medium.

2. The most active extracts were obtained on the soy bean medium, which brought about as much as 50 per cent hydrolysis of the oil in 20 hours.

3. On the bran-wood flour-olive oil medium, the optimum concentration of the oil for lipase production lies between 2.5 and 5 per cent.

4. Lipase production is greatest after three to four days' incubation, or just at the time of complete sporulation.

5. Enzyme production is greatest when the cultures are incubated at 28° C. in a water bath. At this temperature in a hot air incubator, some of the lipase is apparently destroyed by the heat given off by the growing fungus.

6. A ratio of about 7.5 parts of soy bean to 12.5 parts of bran yields the best medium for lipase production.

7. Slightly more lipase is elaborated on a medium containing soy beans than on one containing soy bean meal. Flax seed and cotton seed do not result in as high a yield of lipase, although the meals produced from these seeds serve equally as well as the seeds themselves.

8. The enzyme precipitated from the *Penicillium oxalicum* extract by means of alcohol contained eight and a half times more lipase per unit of protease than did a commercial high lipase trypsin. The activity was low when the enzyme was obtained from large cultures; the dry enzyme prepared from 40 gm. lots of medium showed good activity.

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A NEW SPECIES OF CALAMOPITYS FROM THE AMERICAN DEVONIAN[1]

DALE E. THOMAS

(WITH PLATES II, III)

Calamopitys eupunctata sp. nov.

Pith small, containing medullary tracheids and medullary xylem strands. Circum-medullary strands in a discontinuous ring. Leaf traces single at origin, becoming double farther out in the secondary xylem. All primary xylem strands centrally mesarch. Secondary xylem tracheids with multiseriate bordered pits on both radial and tangential walls. Pits evenly distributed, not grouped. Growth rings distinct; summer wood tracheids radially narrower and with thicker walls than those of the spring wood. Wood parenchyma abundant in the terminal position. Locally branched tracheids. Wood rays commonly uniseriate, frequently biseriate, broader rays occasional. Rays high.

Description of material

The plant material upon which this paper is based was collected by Professor L. C. PETRY, in October, 1924. The find was made in Taughannock Gorge, located on the west shore of Cayuga Lake, in central New York, about 10 miles from the upper or southern end of the lake. Stem fragments of this material were found near the foot of a rock fall on the north side of the gorge, about 100 yards below the main waterfalls. They were in one of the slabs of rock which held *Callixylon* remains described by ARNOLD (1). This slab had fallen from the Cashaqua Shale, exposed some 300 feet above the bed of the stream (18). The Cashaqua Shale belongs to basal Portage, a member of the Upper Devonian. It corresponds to the Sherburne flagstone of Ithaca as classified by WILLIAMS (22).

PETRY made a preliminary examination of sections which he cut from the specimen, and recognized that the material represented an

[1] This paper represents part of a thesis (18) presented to Cornell University, 1932.

undescribed form. Later he turned over to the writer all of this fossil material for preparation and description.

The material when found consisted of two pieces which matched along a plane oblique to the axis of the fossil. The specimen, as reconstructed, measured 75 mm. along its axis and had a maximum diameter of 30 mm. It tapered gradually to a point at one end and was broken obliquely at the other end.

The fossil was inclosed in a calcareous nodule, gray in color, with a slight reddish brown tinge. The fossil itself was black or nearly so, with marcasite filling the lumina of many of the cells. This iron sulphide was especially abundant in the wood rays of the fossil stem and has interfered to some extent with the preparation and interpretation of the material.

While the preservation is generally good for material of this type and age, nevertheless the specimen shows numerous local mineral replacements with a loss of cellular structure. Many of these regions probably represent the filling of cavities of local decomposition which antedated fossilization. These are most frequent in those parts of the stem originally occupied by softer parenchymatous tissues.

The description of this fossil material is based upon microscopic examination of the sections prepared by PETRY and also of a great number of sections prepared by the writer. Two types of slides were prepared,[2] cellulose peels and ground and polished sections. The collection of slides prepared from this stem includes 23 transverse sections, 12 tangential sections, and 14 radial sections. Many of these sections include only fragmentary material.

General anatomical structure

The outstanding features of a transverse section are: (1) the four parabola-like or fanlike groups of secondary xylem which include most of this tissue, (2) the prominent growth rings, and (3) the irregular shape of the pith (fig. 1).

PITH

The pith is rather indefinite as to its outer limits, extending between the wedges of the secondary xylem. The form is dependent

[2] For a description of the methods used in preparing the slides, see THOMAS (**18**), BARNES and DUERDEN (**3**), WALTON (**20, 21**), and KRÄUSEL (**7**).

upon the position of the leaf traces. In transverse section it is in general about 4 mm. long and 1.5 mm. broad, as measured to the circum-medullary primary xylem. It contains both primary xylem strands and a considerable number of more or less isolated tracheids. The parenchyma cells are not so well preserved; mineralization has been very pronounced wherever the cells formerly existed. These are typical parenchyma cells, short and with thin walls (fig. 2). The tracheids of the medullary strands show distinct sculpturing (fig. 3), as do the tracheids of the circum-medullary strands bordering the secondary wood.

The writer considers this stem protostelic, with much parenchyma accompanying the primary xylem. It may be considered as siphonostelic since it has a definite pith with considerable parenchyma in which are located medullary strands and the more or less isolated tracheids. It is in reality a condition intermediate between a typical protostele and a typical ectophloic siphonostele.

Primary xylem

The primary xylem is not a continuous mass of well organized tissue but consists of strands. Some of these strands, the medullary bundles, are located well within the pith; others, the circum-medullary strands, are in contact with the inner ends of the radial rows of secondary xylem elements. Radially broad strands lie between the medulla and each leaf trace. These radiating strands are sheetlike in form, some 10 to 12 tracheids wide in tangential direction, and with rows of secondary tracheids radiating out from each of them.

The circum-medullary primary xylem, in contact with the inner portion of the secondary wood, is 3 to 6 cells thick, an unusually thin layer. The medullary strands which are imbedded in the pith may be from 10 to 20 cells broad. Numerous medullary tracheids are found isolated in the pith; they also occur in small groups of from 3 to 6 elements. A longitudinal section shows that at least some of these medullary tracheids have connections with the medullary strands at a lower level. It is probable that these elements, which appear to be isolated as seen in cross sections, may be ramifications from the primary strands.

The centrally mesarch position of the protoxylem is illustrated in

figure 2, in a medullary bundle. The same condition may also be seen in the leaf trace bundles (figs. 4, 5). These illustrations also show that the centripetal and centrifugal tracheids are approximately the same size.

The protoxylem elements average 22 μ in diameter; metaxylem elements average 40 μ, with a maximum diameter of 55 μ observed. These are somewhat smaller than the tracheids of the secondary xylem, which average 50 μ and have an observed maximum diameter of 85 μ.

The markings on the walls of the protoxylem elements are in many places clearly visible. They are in all cases either annular or spiral (figs. 3, 6). The metaxylem includes only scalariform and pitted elements.

Secondary xylem

The secondary xylem constitutes the major part of the specimen (fig. 1). The arrangement of the secondary wood in four fan-shaped groups is characteristic throughout the specimen. This feature is associated with the outgoing leaf traces. The tracheids of this tissue range in diameter from 30 to 85 μ, with an average of 50 μ. The rows of tracheids are oriented in two ways: (1) in strictly radial rows, with the inner (proximal) portion of the rows well within the pith; (2) most of the rows are arranged in the four fan-shaped groups, the proximal portion of each row curving strongly toward the primary xylem in the radial axis of this group. The wood is traversed by numerous wood rays.

The walls separating the lumina of two adjacent tracheids of the secondary xylem average 9 μ in thickness. The thickness of the interluminal walls of the parenchymatous wood ray cells is less than 2 μ. The pits on the radial walls of the tracheids are evenly distributed. Alternate–multiseriate pitting predominates, with 3, 4, and 5 series the general rule. The pits are elongate in shape, measuring 9×3.5 μ, with the longer axis at right angles to that of the tracheid. The pits are full bordered (fig. 7).

One of the outstanding features of this stem is the tangential pitting, which is as clearly distinct as the pitting on the radial walls. Tangential pits are in shape, size, and arrangement like those of the

radial walls; frequently they are more scattered however (figs. 8, 9). In this stem, pits are present on all tangential walls and not solely on those of the terminal tracheids as in *Ginkgo* (**5**, p. 434) and some conifers.

Tangential pitting has been observed, in rare cases, in Paleozoic stems. It was first reported by SCOTT (**9**) for *Pitys antiqua*. Geologically this feature occurs as low as Middle Devonian, where it was reported by KIDSTON and LANG (**6**) in *Palaeopitys milleri*. Tangential pitting has also been reported in the following Paleozoic gymnosperms: *Callixylon trifilieve*, *C. newberryi* (**2**, **15**), *Volkelia refracta* (**17**), *Mesoxylon multirame* (**12**), and *Bilignea resinosa* (**13**). These are all from the Carboniferous except *Palaeopitys milleri*, which is from the Middle Devonian and *Callixylon trifilieve* which is from the Upper Devonian.

SCOTT (**9**, p. 352) states that the pits do not cover the whole tangential surface of the tracheids in *Pitys antiqua*, nor are they as crowded as the pits on the radial walls. SCOTT (**13**) found the pitting on tangential walls of *Bilignea resinosa* differed from that on radial walls in being more widely scattered and in having much smaller borders. In *Mesoxylon multirame* tangential pitting is found only on a few tracheids. ARNOLD (**2**) reports that the pits on the tangential walls of the tracheids of *Callixylon newberryi* are smaller, more distantly spaced, and not grouped as are the radial pits.

The tangential pitting which resembles most that found in *Calamopitys eupunctata* has been described and photographed by KIDSTON and LANG (**6**) from *Palaeopitys milleri*. Their description applies to *C. eupunctata* except that they found the shape of the pits polygonal; those of *C. eupunctata* are oval. The polygonal shape is due to crowding, of course.

Another distinctive feature of *C. eupunctata* is the presence of branched tracheids. The branching occurs only in the tangential plane; it is frequent in regions where parenchymatous tissue is abundant and in connection with leaf traces (fig. 8). Evidence of tangential branching may be observed also in transverse sections (fig. 10). These show that branched tracheids occur not singly but in radial rows.

The writer has found no reference to branched tracheids in the

literature on fossil or on living gymnosperms. This irregularity in the form of some of the tracheids of the Taughannock specimen is a prominent characteristic in tangential sections.

The so-called "convoluted tracheids" of *Callixylon zalesskyi* described by ARNOLD (1) suggest a remote similarity to the branched tracheids. The convoluted appearance, however, seems not to include branching but only curvature and other distortions of the normal shape. There are a few places in the photomicrograph illustrating this feature where one might suspect branching.

SCOTT and JEFFREY (**14**, fig. 35) include a photomicrograph of a tangential section of *Archaeopitys eastmanii* which also shows a slight similarity to the branched tracheids. They use the term knots in referring to the features which they have illustrated. SCOTT (10), in describing similar "knots" observed in *Mesoxylon lomaxii*, states that in the outer secondary wood the position of a double leaf trace is marked, in tangential section, by the presence of two knots of irregularly interwoven tracheids, inclosing in their meshes the tracheids of the outgoing strand.

The "convoluted tracheids" and "knots" of other investigators may not be analogous to the "branched tracheids," but they are the only structures at all comparable with them.

Growth rings are pronounced in this specimen. The larger sections show four "annual rings." The difference in the tracheids in the spring and summer wood is twofold: a difference in the radial width of the elements and a difference in the thickness of their walls. The summer wood elements average, in radial breadth, 42 μ; those of the spring wood, 74 μ. The interluminal walls of summer wood elements average 10.5 μ; those of the spring wood elements, 8.3 μ. The most striking feature of the growth rings is the occurrence of parenchyma in the terminal position (figs. 11, 12, 13); this feature will be described later.

The presence of growth rings in Paleozoic fossil plants is only occasional although several cases have been reported. Among the species which show annual rings are *Callixylon erianum* (1), *Endoxylon zonatum* (13), *Bilignea resinosa* (13), and *Dadoxylon indicum* (4). The growth rings of the Taughannock specimen resemble most those of *B. resinosa*. This new species, however, differs in that it has

advanced to a higher state in the development of growth rings, with its terminal parenchyma.

The terminal parenchyma cells are radially much narrower, shorter, and with much thinner walls than the tracheids. Figure 13, a radial section, shows the short terminal parenchyma cells with the summer wood tracheids on the left and the spring wood tracheids on the right of the parenchyma cells. The terminal parenchyma constitutes a cylindrical sheet, with numerous patches of summer wood tracheids occupying from 15 to 20 per cent of the area. This cylinder is also interrupted by leaf trace windows. The terminal position of the parenchyma cells is established by the fact that these parenchyma elements are contiguous vertically with the narrow, thick walled, summer wood tracheids, and radially adjacent to the spring wood tracheids.

The presence of terminal parenchyma is the most advanced characteristic of this Upper Devonian stem. While terminal parenchyma is characteristic of some living genera of conifers, such as *Abies*, *Larix*, and *Tsuga*, it has not heretofore been reported in Paleozoic gymnosperms.

SCOTT (**9**) found isolated strands of xylem-parenchyma in *Pitys antiqua*, also in *Mesoxylon multirame* (**12**). JEFFREY (**5**) placed the origin of terminal parenchyma in the Jurassic. He states that parenchyma originated first in the terminal position in gymnospermous woods. Now, even though found much lower stratigraphically, it was found in the primitive position. It was also found in a woody stem with tangential pitting.

The tracheids are extremely variable in length, commonly 400–1000 μ long, the maximum measured being 1875 μ. Tangentially the ends of the tracheids are chisel-shaped. The end walls are perforated by typical bordered pits.

The wood rays are most frequently uniseriate; biseriate rays are common; broader rays are found. Uniseriate rays are commonly 5–9 cells high; broader rays vary from 10 to 25 cells high; one triseriate ray 30 cells high was observed (figs. 14, 15). The wood ray cells are all of one type, typical parenchyma cells. In some cases the wood ray lies between strictly parallel radial sheets of tracheids, a row of tracheids occupying the space between two vertically con-

secutive rays, the ends of the tracheids abutting on the marginal ray cells (fig. 15). Generally, however, the wood ray occupies a space between rows of tracheids which are contiguous above and below the rays. Tangential sections show both relationships.

Leaf traces

The arrangement of the secondary xylem in four fanlike sections is the most obvious feature in a transverse section of this stem. This characteristic is the result of the passing of leaf traces through the secondary xylem. Each of the transverse sections shows the four fans of secondary xylem. These fans include practically all of the secondary wood; only a few rows of tracheids lie in a strictly radial position, not inclined toward one of the circum-medullary protrusions in the axis of a leaf trace (fig. 1).

At no place in any of the sections is a leaf trace closer to the pith than 2 mm. The inner traces are always simple and centrally mesarch (fig. 4). Farther out the primary strand of the trace divides into two bundles (fig. 5). Still farther out and within the secondary xylem the trace assumes a direction perpendicular to the axis of the stem. Here a cross section of the stem shows a longitudinal section of the leaf trace with the two groups of protoxylem (fig. 6). The outermost traces are more complex.

The radial protrusion of circum-medullary primary xylem which extends distally to each leaf trace corresponds to the leaf gap of a siphonostelic stem. In the case of a siphonostele, parenchyma occupies the leaf gap in the axil of the trace; in this case primary xylem follows the outgoing trace. This radial protrusion is clearly shown in connection with the bottom leaf trace in figure 1. These narrow primary xylem wedges contain from 3 to 6 protoxylem points. This primary strand is in contact with an inner simple trace but is separated from the more distal traces by parenchyma tissue. There is no definite proof that a typical reparatory strand is formed but there is evidence favoring this interpretation. The double trace appears at times to have a reparatory strand just proximal to the trace and separated from both the trace and the radial protrusion by parenchyma.

Comparison of Calamopitys eupunctata with other species of Calamopitys

The genus *Calamopitys* was described by UNGER (**19**) in 1856, founded upon *C. saturni* as the type species. Since then the following species have been added: *C. annularis* by SOLMS-LAUBACH (**16**), *C. beinertiana* and *C. fascicularis* by SCOTT (**8, 9**), *C. americana* by SCOTT and JEFFREY (**14**), and *C. radiata* by SCOTT (**13**).

C. americana and *C. eupunctata* have in common a mixed pith, mesarch xylem strands, high multiseriate rays, several rows of alternating pits on the radial walls of the tracheids of the secondary xylem, development of double leaf trace bundle from the single bundle (**11**, p. 209), arrangement of secondary xylem around the leaf trace, and form and character of the ray cells.

C. eupunctata does not check with the description of *C. americana* as reported by SCOTT and JEFFREY (**14**) on the following points:

1. The primary xylem ring of *C. eupunctata* does not show approximate continuity.
2. *C. eupunctata* has primary strands centrally mesarch.
3. Centripetal and centrifugal metaxylem elements of *C. eupunctata* are of the same size.
4. Tracheids of the secondary xylem are slightly larger than those of the metaxylem in *C. eupunctata;* in *C. americana* tracheids of the secondary xylem are smaller than those of the primary strands.

In addition to these exceptions, *C. eupunctata* shows the following characteristics not mentioned by SCOTT and JEFFREY (**14**) nor by SCOTT (**11**):

1. Arrangement of the bulk of the secondary xylem in four fan-shaped wedges.
2. Tangential pitting on the walls of all tracheids of the secondary xylem.
3. Distinct growth rings.
4. Abundant terminal parenchyma.
5. Presence of branched tracheids.
6. Wood rays commonly uniseriate.

Many of the items contrasted are quantitative and other characteristics observed in only one of these species may have been present in both species but observed in only one. The fact that most

of the points in which these two species are in disagreement have to do with the secondary xylem is significant.

C. eupunctata resembles *C. saturni* in having a discontinuous circum-medullary primary xylem and in having centrally mesarch primary strands. It differs from *C. saturni* in having medullary tracheids and medullary xylem strands, and in lacking the outward dilation of its wood rays. These two species differ also at all points in which the new species and *C. americana* differ, except in the form and arrangement of the circum-medullary bundles.

This new species has wood rays more like those of *C. annularis* than like those of *C. americana*, at least in so far as width is concerned. It is more closely allied with these three species than with *C. fascicularis* and *C. beinertiana*, the *Eristophyton* species of ZALESSKY (**23**). *C. eupunctata* shows no specific affinities with *C. radiata*, as described by SCOTT (**13**).

Summary

1. *C. eupunctata* shows all of the generic characteristics of *Calamopitys*, as summarized by SCOTT (**11**). It has a small pith surrounded by a ring of mesarch primary strands; it has a leaf trace which is single at its origin but divides farther out; it has considerable secondary xylem; the secondary and metaxylem tracheids have multiseriate bordered pits.

2. The characteristics which distinguish it from the other species of this genus have to do almost entirely with the secondary xylem. It seems expedient to classify this species under the genus *Calamopitys*. Even though the secondary xylem shows marked variations from that of other species of this genus, these are considered as characteristics of specific value rather than of generic value.

The writer wishes to express his appreciation to Cornell University for use of laboratory and equipment; to Professor L. C. PETRY for the excellent fossil material and for frequent good counsel; to Professors A. J. EAMES, C. M. NEVINS, and L. W. SHARP, and to Mr. D. G. CLARK and Mr. H. T. SCOFIELD for valuable suggestions and assistance.

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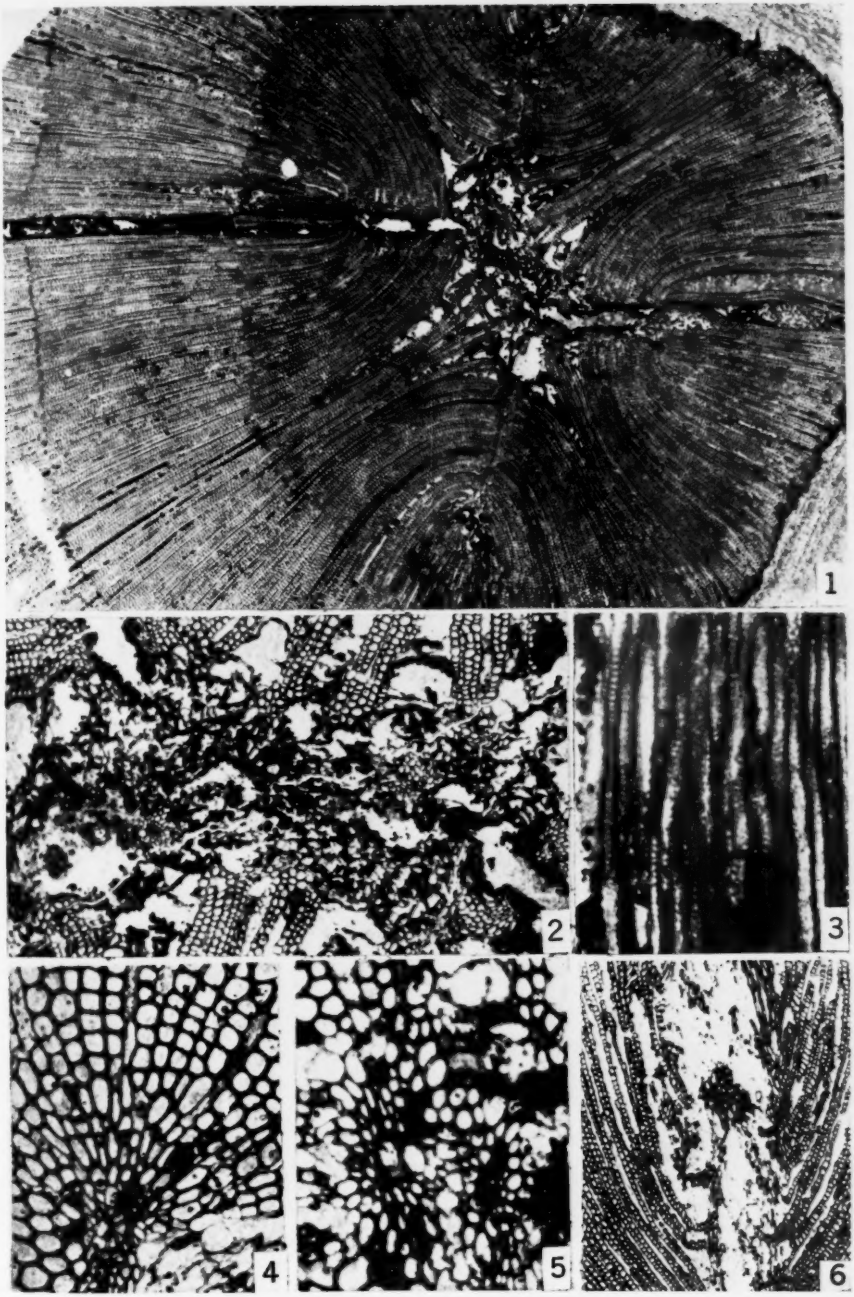

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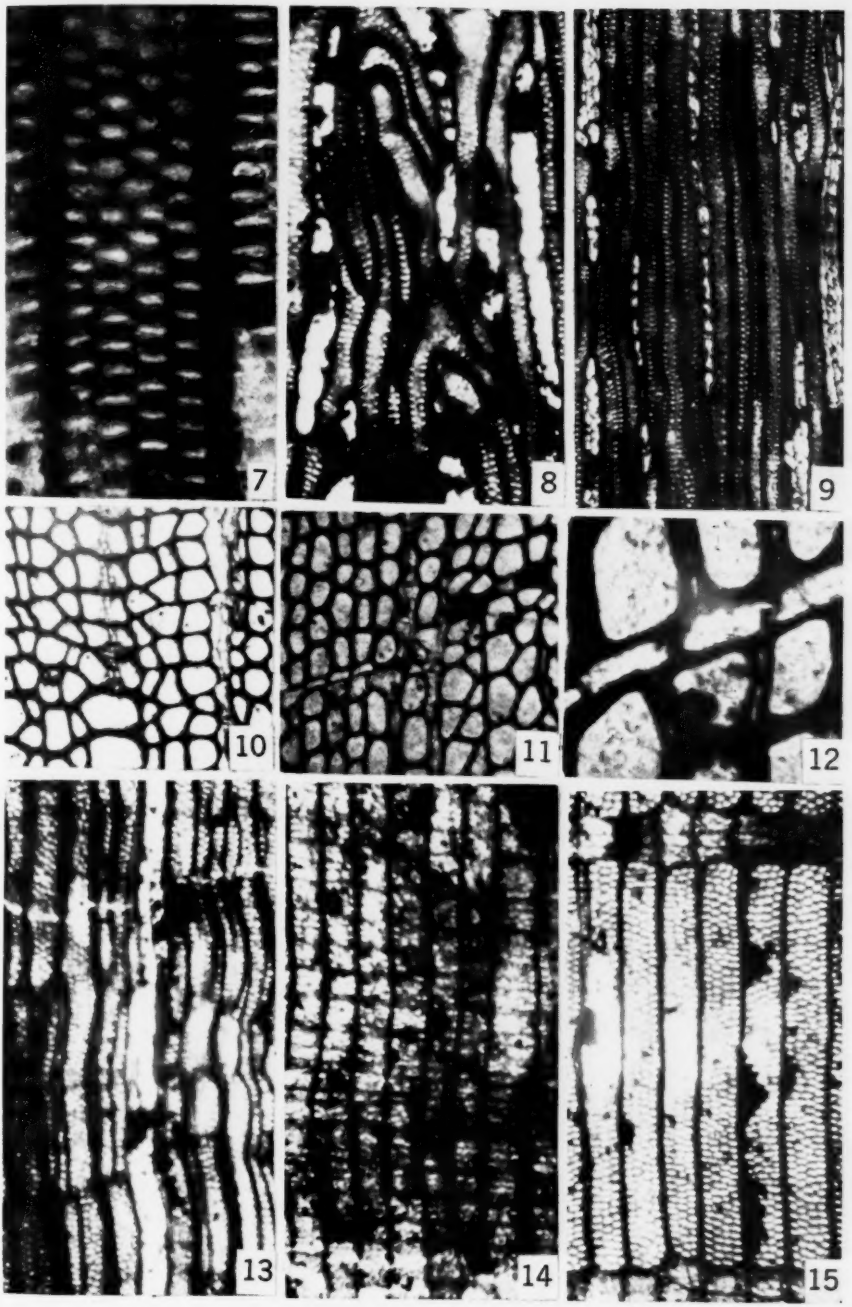

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EXPLANATION OF PLATES II, III

All of the figures are photomicrographs from untouched negatives.

FIG. 1.—General transverse section showing pith, arrangement of secondary xylem, position of leaf traces, and growth rings. ×7.

FIG. 2.—Central part of fig. 1 enlarged to show pith, medullary and circummedullary strands, and arrangement of proximal portion of secondary xylem. ×25.

FIG. 3.—Radial section through medullary bundle showing sculpturing of elements. ×100.

FIG. 4.—Transverse section showing simple leaf trace. ×100.

FIG. 5.—Transverse section showing double leaf trace with two protoxylem groups. ×100.

FIG. 6.—Transverse section (cellulose-peel) showing more advanced stage of double trace; section through primary bundle of trace nearly longitudinal. ×15.

FIG. 7.—Radial section showing pitting of tracheids of secondary xylem. ×450.

FIG. 8.—Tangential section showing branched tracheids accompanied by numerous wood rays. ×80.

FIG. 9.—Tangential section showing wood rays of various widths and heights; pitting on tangential walls of tracheids. ×100.

FIG. 10.—Transverse section showing pitted radial bands crossing some tracheids which are tangentially broad; each band represents the crotch of a branched tracheid. ×100.

FIG. 11.—Transverse section through growth ring; terminal parenchyma running diagonally from left to right; summer wood elements above parenchyma. ×100.

FIG. 12.—Transverse section showing portion of fig. 11 enlarged; summer wood elements above parenchyma. ×450.

FIG. 13.—Radial section showing vertical row of short, thin walled parenchyma cells in terminal position, with summer wood elements on left, spring wood elements on right. ×100.

FIG. 14.—Radial section showing exceptionally high wood ray; shape of ray cells and pits visible. ×100.

FIG. 15.—Radial section showing row of tracheids abutting on wood ray above and below; both rays are low. ×100.

MACROSPOROGENESIS AND DEVELOPMENT OF THE EMBRYO SAC OF LILIUM HENRYI[1]

D. C. COOPER[2]

(WITH PLATES IV, V AND NINE FIGURES)

Until recently, the type of development of the embryo sac in the Liliaceae has been considered one of the simplest to be found among the angiosperms. According to the common conception of the so-called "lily" type, the archesporial cell without division becomes the macrospore mother cell. As a result of three nuclear divisions and later cell divisions, a typical 8-nucleate, 7-celled embryo sac is formed. BAMBACIONI (1, 2), however, in his study of the development of the embryo sac of *Fritillaria persica*, found that four nuclear divisions actually intervene between the archesporial cell and the egg. In the course of the third division, three of the four nuclei formed as a result of the second division migrate to the chalazal end of the embryo sac, leaving one nucleus in its micropylar region. All the nuclei then undergo mitosis. The three chalazal spindles unite to form a common spindle which bears 3n chromosomes, whereas the micropylar spindle bears n chromosomes. As a result of this division the embryo sac is again 4-nucleate, two nuclei being haploid and two triploid. One further nuclear division occurs. BAMBACIONI and GIOMBINI (3) describe a similar process in the formation of the female gametophyte of *Tulipa gesneriana*, and later BAMBACIONI-MEZZETTI (4) reports the same type of embryo-sac development for *Lilium bulbiferum*, *L. candidum*, and *Tulipa praecox*. The writer (6), in a preliminary paper, briefly described a

[1] Papers from the Department of Botany and the Department of Genetics (no. 184), Agricultural Experiment Station, University of Wisconsin. Published with the approval of the Director of the Station.

[2] Since this paper was submitted for publication, six other species of *Lilium* have been examined and a type of embryo sac development similar to that herein described was observed. The species examined were *L. auratum* Lindl. var. *platyphyllum* Hort., *L. elegans* Thunb., *L. martagon* Linn., *L. pardalinum* Kellogg, *L. philippinense* Baker, and *L. tenuifolium* Fisch. Dr. DONALD A. JOHANSEN, Stanford University, in a private communication reports the same type of development as occurring in *L. michiganense* and *L. parryi* Watson.

similar method of embryo-sac formation in *Lilium henryi* and noted that this method is also found in *L. speciosum*, *L. philadelphicum*, and *L. longiflorum* var. *eximium* (*L. harrisii*).

Material and methods

Buds of various ages, open flowers, and young fruits of *Lilium henryi* Baker were collected from plants growing in the gardens of the Department of Horticulture at the University of Wisconsin. The ovaries were dissected out, cut transversely in small pieces, and either fixed in Carnoy's solution or dipped in that solution for a few seconds and then placed in Karpechenko's modification of Navashin's fluid. Good preparations showing stages in the development of the embryo sac were obtained from material in both fixatives. Material of *L. regale* Wilson was similarly fixed for a comparative study. The material was sectioned at 12–18 μ and stained in dilute Delafield's haematoxylin.

Preparations of ovaries of *Lilium* used for class work in the Department of Botany were also examined. These include material of *L. speciosum* Thunb., *L. philadelphicum* Linn., and *L. longiflorum* Thunb. var. *eximium* Nichols (*L. harrisii* Carr.). The history of embryo-sac development was consistent in all the material examined. The figures and description contained herein refer to *L. henryi* unless otherwise stated.

Investigation

Development of embryo sac

In *L. henryi* the primary archesporial cell functions as the embryo sac mother cell. During the heterotypic prophases this cell is about twice as long as broad and is somewhat flattened longitudinally, the nucleus being in the mid-region of the cell. The axis of the spindle of the first meiotic division is approximately longitudinal (fig. 10).[3] Twelve pairs of chromosomes can be counted on the equatorial plate. No cell plate is formed after this division. The second division takes place almost immediately, binucleate stages being relatively scarce in the preparations. Twelve chromosomes can be seen on each of the two equatorial plates in figure 11. The axes of the homoeotypic spindles may be parallel or at an oblique angle to each other. Coulter (7) reported that the micropylar spindle of *L. philadelph-*

[3] See plate IV, facing page 354.

icum in this division is transverse, the antipodal one longitudinal. His figures, however, indicate that he was describing the third rather than the second division, since a greater number of chromosomes appear on the chalazal spindle.

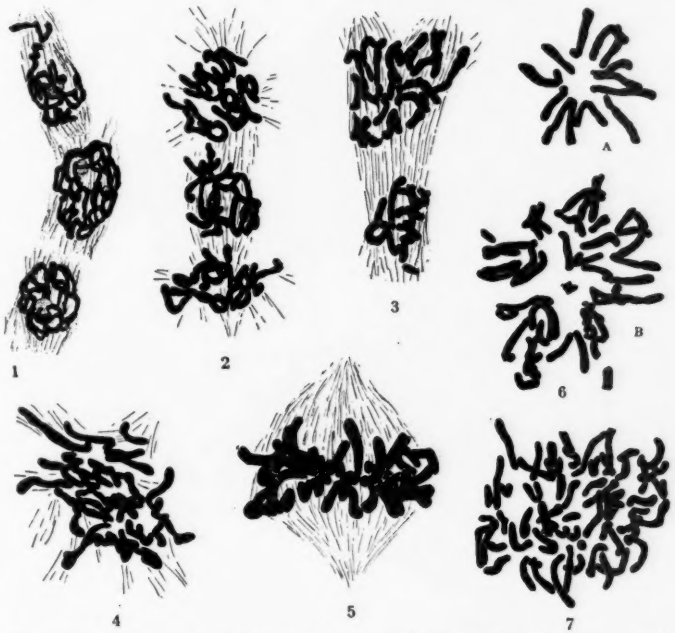
Four nuclei are formed as a result of the homoeotypic division (fig. 12). These nuclei usually lie approximately in a row, evenly distributed along the length of the sac. The micropylar nucleus remains at that end of the sac and the other three nuclei migrate to positions near the opposite end (fig. 13). All four now prepare to divide (fig. 14). SARGANT (**11, 12**) noted indications of spindle fibers surrounding the well differentiated naked spiremes in the 4-nucleate embryo sac of *L. martagon*. These spiremes were always arranged in the same way: a single nucleus near the micropyle and a group of three at the chalazal end. Since she was unable to find 8-nucleate embryo sacs in the neighboring ovules, she did not consider that the division thus indicated had any special significance. Her figure 36 shows the four nuclei in a late spireme with a bipolar spindle at the micropylar end of the embryo sac and a tripolar one at the opposite end. This is probably a prophase stage of the third division.

The nucleus at the micropylar end of the embryo sac of *L. henryi* divides in the usual manner. The division spindle is often in a transverse position (figs. 16, 18, 20, 21, 23, 24, 26), but it may be longitudinal (figs. 19, 27) or at an oblique angle (figs. 17, 25) to the long axis of the sac.

The three chalazal nuclei form spiremes (fig. 13), their membranes disappear (fig. 14), and soon a spindle is formed for each nucleus (text fig. 1; figs. 15, 16). In the chalazal portion of the embryo sac shown in figure 1 and figure 15, the chromosomes of each nucleus are arranged in a discontinuous spireme and the nucleolus is still present in the central region. The spindles formed in connection with these three nuclei now unite in such a manner that a single multipolar spindle is formed (fig. 4) which ultimately becomes bipolar (fig. 5). During this rearrangement the chromosomes become so shifted and rearranged (text figs. 2, 3; figs. 17–22) as to form a common equatorial plate. COULTER (**7**) noted multipolar spindles in *L. philadelphicum* (his figs. 9 and 11), but thought this to be an unusual, possibly a pathological condition.

The single equatorial plate now contains 36 chromosomes (fig.

6 *b*, fig. 24). The spindle is usually arranged longitudinally in the embryo sac (figs. 23, 25, 26), but may be transversely oriented (fig. 24). The embryo sac now again contains two spindles (figs. 23–26), but at this stage there are 12 chromosomes on the micropylar and 36 on the chalazal spindle (fig. 24). COULTER's figures 3, 4, 5, 9, 10, 11, and 12 show more chromosomes on the chalazal spindle than are



FIGS. 1–7.—Figs. 1–3, stages in fusion of three chalazal spindles in embryo sacs whose nuclei are undergoing third division. Fig. 4, polar view of multipolar spindle in chalazal end. Fig. 5, lateral view of bipolar spindle formed as result of fusion of spindles. Fig. 6: *A*, polar view of micropylar equatorial plate showing 12 chromosomes; *B*, polar view of chalazal equatorial plate with 36 chromosomes. Fig. 7, polar view of equatorial plate in endosperm cell; 60 chromosomes (5n) present. All ×750.

to be found on the micropylar spindle of what he considered the homoeotypic division. Four nuclei are again present at the end of this third division; two large nuclei lie in the chalazal region, and two smaller ones in the micropylar region (figs. 27, 28). The spindles of the third division are more or less persistent. GUIGNARD (8) noted a curious difference between the numbers of chromosomes present

respectively in the micropylar and chalazal nuclei in the embryo sac of *L. candidum*. MOTTIER (9) likewise observed a greater number of chromosomes on the chalazal spindle, but considered this condition to be due to an increased amount of chromatic material formed as a result of growth.

Up to this time the cytoplasm has been finely vacuolate, and no large central vacuole is present in the mid-region of the sac. The sac now grows and elongates, coming to be about four times as long as broad. Vacuoles (fig. 28) appear in the cytoplasm in the mid-region; these may unite to form a large central vacuole. In some instances, however, the central vacuole is not conspicuous until after the last nuclear division (fig. 38).

A densely staining mass of cytoplasm appears at each end of the spindles of the third division, in close contact with the nuclei (fig. 27). These masses are more prominent and have the appearance of a felted pad applied to one side of the nucleus in the case of *L. speciosum* (fig. 28). Remnants of the spindles remain in the cytoplasm between these pads. COULTER showed the remnants of such spindles in his figure 6. He considered this the 4-nucleate stage resulting from the homoeotypic division, but because of the difference in the size of the nuclei it appears probable that he had a 4-nucleate stage following the third division.

Three of the four nuclei now present, the two smaller (n) ones at the micropylar end of the embryo sac and one of the 3n nuclei in the chalazal region, undergo typical mitoses (fig. 29). Many more chromosomes are present on the chalazal than on either micropylar spindle. COULTER in his figures 13, 14, and 15 showed more chromosomes on the chalazal spindles than on the micropylar spindles of the last division.

The other 3n nucleus, near the chalazal end of the embryo sac, undergoes a more or less abortive division. Although there seems to be no regular formation of chromosomes, a spindle is formed and granules or masses of chromatic material pass to each pole. This nucleus is in a reticulate condition at the time its sister nucleus contains a spireme (fig. 30). Later a spindle is formed, and chromatic material or aggregations of this material are to be seen on the spindle (figs. 31–33). In some instances bodies resembling chromosomes are present (fig. 33). These pass irregularly to the poles and two small,

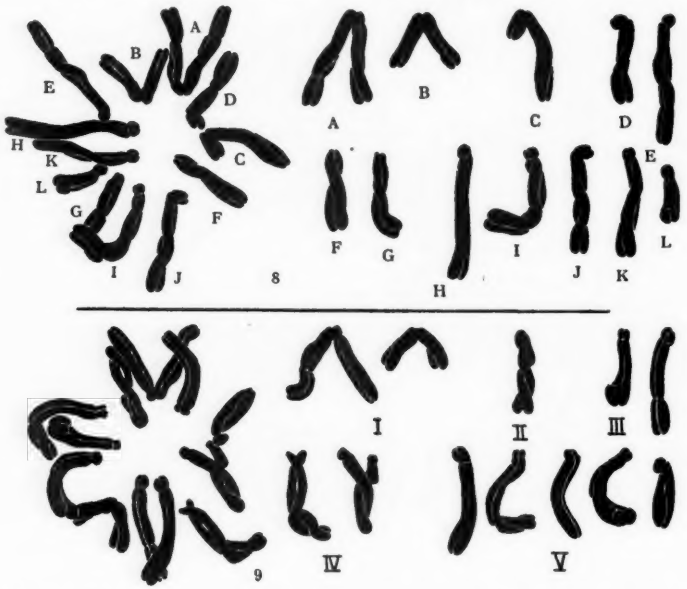
somewhat irregularly shaped nuclei are formed simultaneously with the development of normal nuclei in other portions of the embryo sac (figs. 34, 35). There is no evidence that direct division of this second chalazal nucleus ever occurs. An examination of SARGANT's (11) figures of amitosis in *L. martagon* shows stages similar to those herein described. MOTTIER likewise figured a spindle in connection with the abortive division of the chalazal nucleus of the same species.

In one case observed, only three spindles were to be seen at the time of this last division (fig. 36). In this instance, however, about 70 chromosomes could be counted on the chalazal spindle (fig. 37). This would seem to indicate that in this instance the two chalazal spindles of the last division, like those of the third division, had united into a common spindle.

During the prophases of the fourth division, the felted pads found at the sides of the nuclei spread out into perinuclear zones, so that, at the equatorial plate stage, each spindle is practically surrounded by a zone of dense cytoplasm (fig. 29). The remnants of the spindles of the preceding division persist between the respective perinuclear zones. At the conclusion of the fourth division the four nuclei at each end of the embryo sac are held together by two prominent spindles and a less prominent one (figs. 34, 35). Cell plates are formed across these three spindles in such a manner that three cells are formed at each end of the sac, leaving two nuclei, one small (n) and one large (3n), in the large central cell (figs. 35, 38). The formation of a cell plate across the spindle nearest the chalazal end of the sac is further evidence that the abortive division at this end is essentially mitotic rather than amitotic (fig. 34). COULTER in his figures 14 and 15 showed remnants of the spindles of the preceding division in *L. philadelphicum* still extant at the anaphase and telophase stages of the final division.

Stages in fertilization (fig. 39) show the union of one male gamete nucleus with the egg nucleus to form the zygote (figs. 39, 40). The other male gamete nucleus unites with the two polar nuclei, one large (3n) and one smaller (n), to form the primary endosperm nucleus (figs. 39, 41). The male gamete nucleus may be easily identified by its shape. SAX (13) figured the fusion of one large and two smaller nuclei in the formation of the endosperm mother cell of *Fritillaria pudica*, as does also NOTHNAGEL (10) in the case of

Lilium martagon. BLACKMAN and WELSFORD (5) figure a similar fusion of nuclei in both *L. martagon* and *L. auratum*. Sixty chromosomes (5n) are present on the equatorial plates of dividing endosperm nuclei (fig. 7). Such a number is to be expected as a result of the union of nuclei herein described. The relationship between embryo (2n), endosperm (5n), and nucellus (2n) differs markedly from the 2-3-2 relationship usually found in angiosperms.



FIGS. 8, 9.—Fig. 8, polar view of haploid equatorial plate in third nuclear division in embryo sac. At right, chromosomes *A–L* assorted into 5 groups according to size and shape. Fig. 9, polar view of haploid equatorial plate in last nuclear division in embryo sac. At right chromosomes arranged in 5 groups according to size and shape. All ×1450.

CHROMOSOME MORPHOLOGY

Polar views of the haploid equatorial plates of the third and fourth nuclear divisions in the formation of the embryo sac are ideal for a study of the shape and individuality of the chromosomes. Five types of chromosomes are distinguishable at this stage. The first is a V-shaped element with the spindle-fiber attachment zone in its midportion (fig. 9 I). The larger of the two chromosomes of this type

shows approximately a 20 per cent difference in length between its arms (fig. 8 *A*). The longer arm has a secondary constriction about one-third its length from the distal end. The shorter arm has a similar constriction closely adjacent to the attachment zone. The smaller V-shaped chromosome has a median attachment region (fig. 8 *B*). The second type of chromosome, of which only one occurs, is J-shaped (fig. 9 II). The attachment region is so located that one arm is approximately three times the length of the other (fig. 8 *C*).

A third type of chromosome has a rather large lobe beyond the attachment region and a secondary constriction about one-third of its length from the opposite end (fig. 9 III). The two chromosomes of this type differ markedly in length (fig. 8 *D*, *E*). A fourth type (fig. 9 IV), represented by two chromosomes, is rod-shaped with a small subspherical head end (fig. 8 *F*, *G*). A fifth type has a long shaft with a rounded lobe beyond the attachment region (fig. 9 V). The five chromosomes of this pattern range in length so that the longest is twice the length of the shortest (fig. 8 *H–L*).

Summary

1. The egg is removed from the macrospore mother cell of *Lilium henryi* by four divisions instead of three as has usually been described for members of this genus.

2. The nucleus of the macrospore mother cell and its daughter nuclei pass through the heterotypic and homoeotypic divisions respectively without cell division, so that the embryo sac is 4-nucleate.

3. Three of these nuclei pass to the chalazal end of the embryo sac; all four nuclei divide simultaneously.

4. The spindles of the three chalazal nuclei unite and a single bipolar spindle bearing 36 chromosomes is formed.

5. As a result of the third division the embryo sac is again 4-nucleate, two haploid nuclei being at the micropylar end and two triploid nuclei at the chalazal end.

6. Three of these four nuclei, the two with n chromosomes and one with 3n, pass through typical mitoses; the other chalazal nucleus divides in an abortive manner.

7. The cells of the embryo sac are formed as a result of cell-plate formation across the spindle of this last (fourth) division as well as across the persistent spindles of the preceding division.

8. In the process of fertilization one male gamete nucleus unites with the egg nucleus to form the zygote nucleus (2n) and the other male gamete nucleus unites with the two polar nuclei, one small (n) and one large (3n), to form a 5n primary endosperm nucleus.

9. Sixty chromosomes are present on equatorial plates of dividing endosperm nuclei.

10. An examination of preparations showing embryo-sac development in *L. speciosum*, *L. philadelphicum*, *L. longiflorum* var. *eximium* (*L. harrisii*), and *L. regale* reveals a history similar to that here described for *L. henryi*.

11. The history of embryo-sac development reported here for five species of *Lilium* is essentially the same as that described by BAMBACIONI for other members of the Liliaceae. It is very probably the characteristic lily type.

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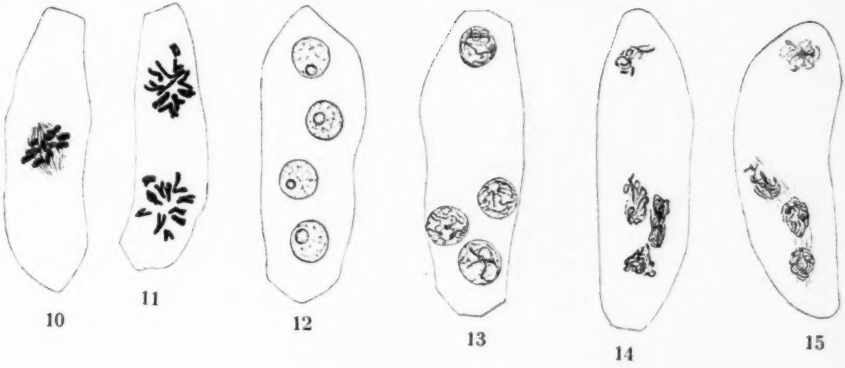
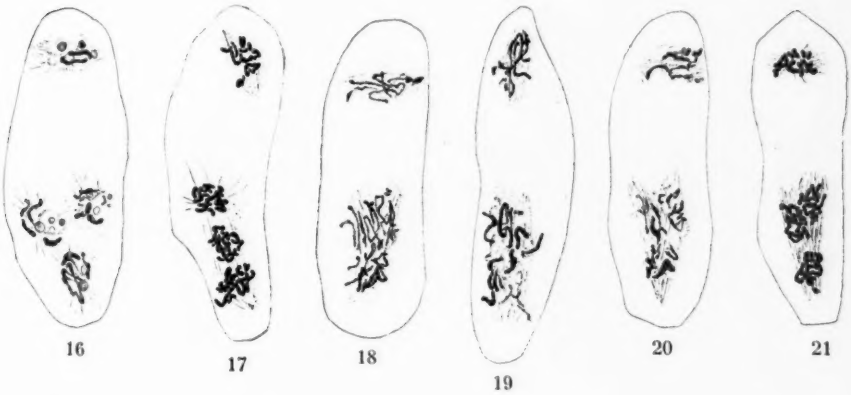
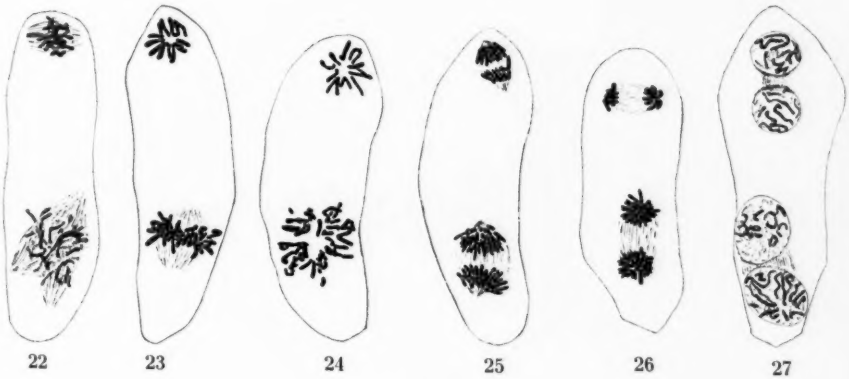
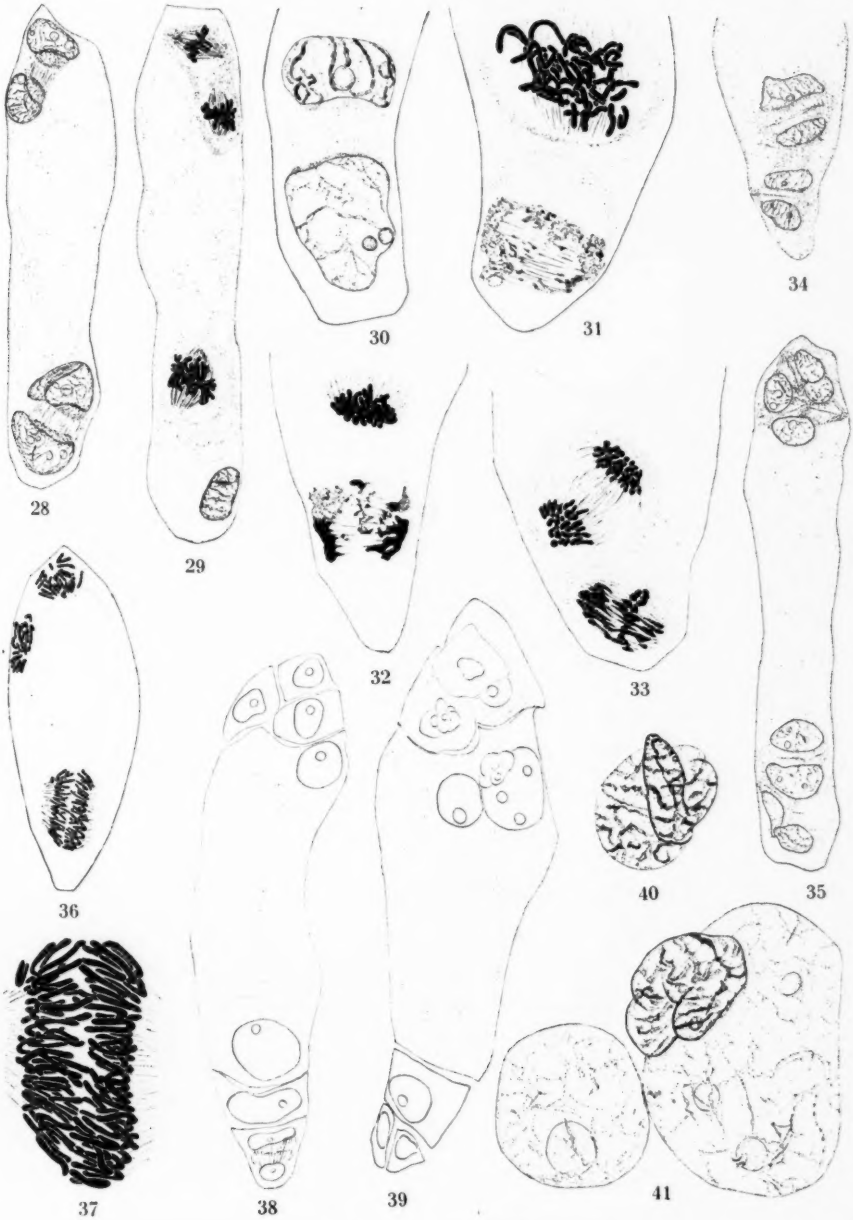






COOPER on LILIUM



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EXPLANATION OF PLATES IV, V

All figures unless otherwise stated were drawn at a magnification of 480× and are reduced approximately one-half in reproduction. All figures are so arranged that the micropylar end of the embryo sac is toward the top of the page.

PLATE IV

FIG. 10. Macrospore mother cell of *L. henryi*. Lateral view of heterotypic equatorial plate.

FIG. 11. Polar view of homoeotypic equatorial plates.

FIG. 12. Four-nucleate embryo sac after homoeotypic division.

FIG. 13. Same, later stage. Three nuclei migrated to chalazal region of sac.

FIG. 14. Same. Nuclear membranes disappeared.

FIG. 15. Early spindle development, third nuclear division.

FIGS. 16–22. Stages in union of the three chalazal spindles. Orientation of micropylar spindle also shown.

FIG. 23. Bipolar chalazal spindle.

FIG. 24. Polar view of two spindles of third division, with 12 chromosomes present on micropylar and 36 on chalazal spindle.

FIGS. 25, 26. Anaphase and telophase stages of third division.

FIG. 27. Four-nucleate embryo sac after third division, showing two small and two large nuclei and persistent spindles.

PLATE V

FIG. 28. *L. speciosum*, somewhat later stage than figure 27, showing cytoplasmic pads on adjacent surfaces of nuclei as well as persistent spindles.

FIG. 29. *L. henryi*, equatorial plates, last (fourth) nuclear division in embryo sac.

FIGS. 30–33. Stages in abortive division of chalazal nucleus. ×850.

FIG. 34. Cell-plate formation at chalazal end of embryo sac.

FIG. 35. Cell-plate formation at both ends of embryo sac.

FIG. 36. Fourth nuclear division, showing large spindle in chalazal region.

FIG. 37. Chalazal spindle from figure 36. About 70 chromosomes passing to each pole. ×1450.

FIG. 38. Eight-nucleate, 7-celled embryo sac.

FIG. 39. Embryo sac showing nuclear unions.

FIG. 40. Male gamete nucleus closely appressed to egg nucleus (from fig. 39). ×2900.

FIG. 41. Male gamete nucleus in close association with two polar nuclei (from fig. 39). ×2900.

ANATOMY OF THE SEEDLING OF ASPARAGUS OFFICINALIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 466

NAOMI MULLENDORE

(WITH FORTY-FOUR FIGURES)

Introduction

Of the works published concerning *Asparagus officinalis* L., some, such as those of ROBBINS (11) and JONES and ROSA (9), have dealt chiefly with the cultural and commercial aspects rather than with the morphological ones. BORTHWICK (4), ROBBINS and BORTHWICK (12), COOLEY (5), and BLASBERG (3) have used morphology largely as a basis for physiological study. Some of these have dealt with limited phases, BORTHWICK (4) and ROBBINS and BORTHWICK (12) with germination; COOLEY (5) with reserve cellulose of the seed. BLASBERG (3) has given some attention to anatomy. His work included growth habit, seedling development, root structure, and stem structure. SCHOLZ (13) has discussed morphology but has not given the detail of the development of the lateral roots and the root-stem transition. ARBER (1) has discussed the cladophylls and leaves. POESE (10) has dealt with the concentric and collateral vascular bundles of the rhizome and stem. BERNÁTSKY (2) has explained the development of the rhizome and the succeeding spears, and something of general structure. ZWEIGELT (17) has compared the members of the Asparagoideae on an anatomical basis.

The present study concerns itself with the details of the anatomy of the seedling, only parts of which have been reported previously.

Material and methods

In April, 1932, seeds of asparagus of the Mary Washington variety were obtained from Coker's Pedigreed Seed Company of Hartsville, South Carolina. To hasten germination, the seeds, after sterilization in 0.25 per cent Uspulun solution, were soaked in distilled water for three days in an incubator at approximately 30° C., the optimum

pre-germination time and temperature for soaking recommended by BORTHWICK (**4**). Although many seeds are injured by long soaking, especially at low and high temperatures, COUPIN (**6**) found asparagus seeds strongly resistant to immersion in water. Most of these seeds were planted out-of-doors in a loamy garden soil. To observe germination more closely, some were placed on moistened filter paper in sterilized petri dishes.

After germination began, seedlings were removed at intervals, killed and fixed chiefly in formalin-alcohol and in formalin-acetic-alcohol. Better fixations were obtained in the latter.

Following a suggestion of Dr. H. E. HAYWARD, it was found that heat would loosen the embryos from the soaked seeds so that they could be readily extracted. This made it possible to obtain the embryo from the hard endosperm more easily and with less injury than by dissection. Most of the embryos were obtained by heating in water to a temperature slightly below the boiling point, although no apparent injury resulted from heating the seeds to the boiling point.

The usual procedure for the paraffin method was followed, except that, to soften the lignified tissues, older parts of the plant were treated with 48 per cent hydrofluoric acid from one to five days before dehydration. A modification of the n-butyl alcohol method (**16**) was used in dehydrating these tissues.

Sections were cut 10 μ thick and stained in a modification of Flemming's triple stain. Detailed drawings were made by aid of a camera lucida and a microprojector.

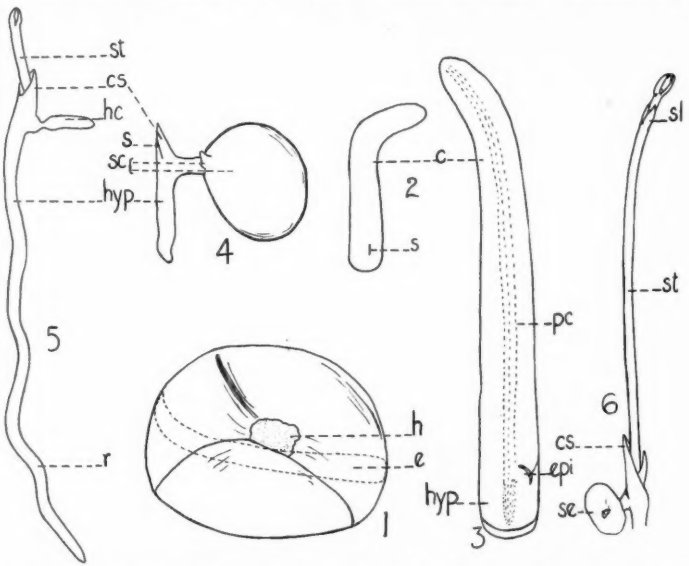
Investigation

SEED AND EMBRYO

The ripe seed, covered with a black coat, the surface of which is finely rugose, is somewhat spherical or subglobose, rounded on the side away from the hilum, but flattened somewhat along one side of the hilum, producing a triangularly pyramidal effect on that side of the seed (fig. 1).

Most of the mature seed is composed of a hard cartilaginous endosperm with walls of hemicellulose (**12, 5**). The slender embryo, 3–4 mm. long, consisting chiefly of the haustorial cotyledon, is imbedded in the endosperm (fig. 1). There is generally a slight curve in the

distal portion of the cotyledon, although some very short embryos are nearly straight. The embryo is blunter at the basal end, tapering gradually to the distal end of the cotyledon. There is a longitudinal slit in the cotyledon about 0.25 mm. in length, located just above the cotyledonary plate (fig. 2). Through this slit, the margins of which represent the edges of the cotyledonary sheath, the shoot will emerge. The tissues of the cotyledon distal to the slit consist of an



FIGS. 1–6.—Fig. 1, seed showing position of embryo. Fig. 2, embryo dissected from seed. Fig. 3, diagram of longitudinal section of embryo. Fig. 4, young seedling showing conelike development of cotyledon. Fig. 5, seedling with haustorial portion of cotyledon dissected from seed. Fig. 6, part of older seedling (*h*, hilum; *e*, embryo; *s*, slit; *sc*, seed coat; *hyp*, hypocotyl; *epi*, epicotyl; *c*, cotyledon; *pc*, procambial strand; *r*, root; *cs*, cotyledonary sheath; *hc*, haustorial part of cotyledon; *sl*, scale leaf; *st*, stem; *se*, seed).

outer layer of papillate, densely protoplasmic, glandular cells; a region of six to ten rows of parenchymatous cells; usually three, sometimes four, procambial strands; and a central parenchyma. The epicotyl is a hemispherical mass of embryonic tissue at the base of the cotyledon within the small chamber below the slit (fig. 3). The short hypocotyl has five or six xylem and as many phloem strands alter-

nately arranged, surrounding a pith. The xylem is generally not lignified. A single layer of pericyclic cells surrounded by the endodermis, ten to fifteen layers of cortical cells, and a single layer of epidermal cells complete the structure of the hypocotyl.

Germination and young seedling

The tip of the hypocotyl lies very near the margin of the seed coat. Upon germination it emerges, tearing out a rounded disc of the seed coat, which, however, remains attached at one point. The basal portion of the cotyledon bearing the slit also emerges and enlarges so that it appears as a conelike structure which incloses the epicotyl (fig. 4). As development proceeds, there appears on the axis inside the cotyledonary sheath the first scale leaf. The next internode elongates appreciably so that the apical meristem and the leaf primordia it bears are brought well above the cotyledonary sheath and the first leaf (figs. 5, 6). The second and third leaves may exhibit a one-half phyllotaxy; the upper leaves are arranged in the two-fifths phyllotaxy. Growth of the parenchymatous cells of the haustorial portion of the cotyledon, by absorption of the surrounding endosperm, proceeds until the cotyledon almost fills the seed coats.

Hypocotyl and primary root

In the hypocotyl there are five or six protoxylem strands alternating with as many phloem strands. Metaxylem is differentiated toward the center surrounding a small pith and can be found in seedlings in the earliest stages of germination (fig. 7). Extensions from the lower portion of the hypocotyl develop into the primary root and possess the same arrangement of tissues. As the root matures the metaxylem differentiates at the central region until no parenchyma is left. Thus is formed the exarch radial protostele (fig. 8). The protoxylem consists of small thick-walled cells with spiral thickenings. The phloem is composed of sieve tubes, many small companion cells, and phloem parenchyma. The pericycle is limited to a single layer of large thin-walled cells. The endodermis has, at first, only small thickenings on the radial walls. Later the thickenings may extend to the inner tangential walls. The cortex consists of eight to twelve layers of cells, the outermost layer of which becomes suberized.

Development of the root axis is produced by two histogenic layers. This agrees with TREUB's findings (**14**) and represents a variation from type 2 of JANCZEWSKI (**8**). In the root tip in the Liliaceae TREUB found only two groups of distinctly separate initials: (1) the initials of the plerome, which are usually very few in number; and (2) a group of many cells outside of these, which serve as common initials for periblem, dermatogen, and cap (fig. 9). These latter ini-

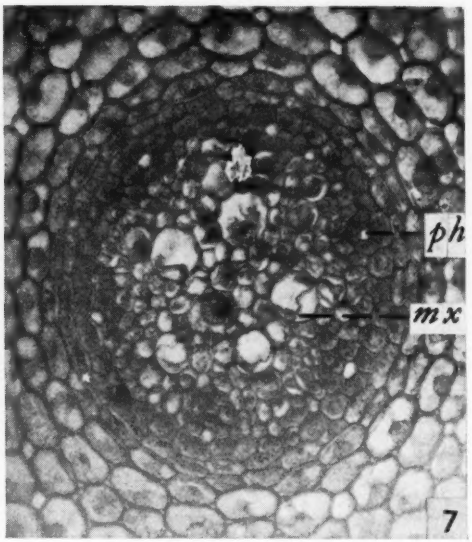
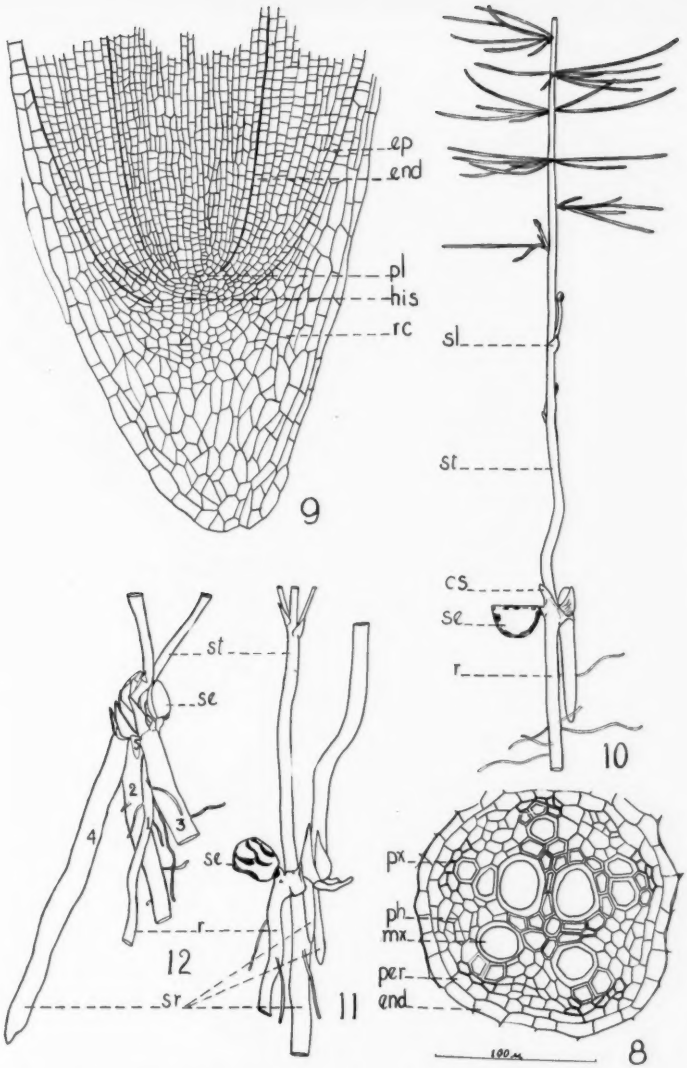


FIG. 7.—Transverse section of stelar region of primary root 1 mm. from root tip (*ph*, phloem; *mx*, metaxylem).

tials give the appearance of a general meristematic region from which new cells are derived with little regularity. Although rows of cells of the cortex and the epidermis may be traced into this region, the rows of cells of the cap are soon indistinguishable owing to irregular divisions of cells.

The mature primary root, less than 1 mm. in diameter and rarely more than 2 dm. in length, may persist throughout the first growing season, although its functions may largely be replaced by those of the large storage roots. The latter arise, apparently without any



FIGS. 8–12.—Fig. 8, transverse section of exarch, radial protostele of primary root. Fig. 9, median longitudinal section of primary root. Figs. 10–12, portions of seedlings to show arrangements of parts (*ph*, phloem; *px*, protoxylem; *mx*, metaxylem; *per*, pericycle; *end*, endodermis; *ep*, epidermis; *rc*, root cap; *pl*, plerome; *his*, histogen for cortex, epidermis, and root cap; *se*, seed; *cs*, cotyledonary sheath; *st*, primary stem; *r*, primary root; *sl*, scale leaf; *sr*, storage roots).

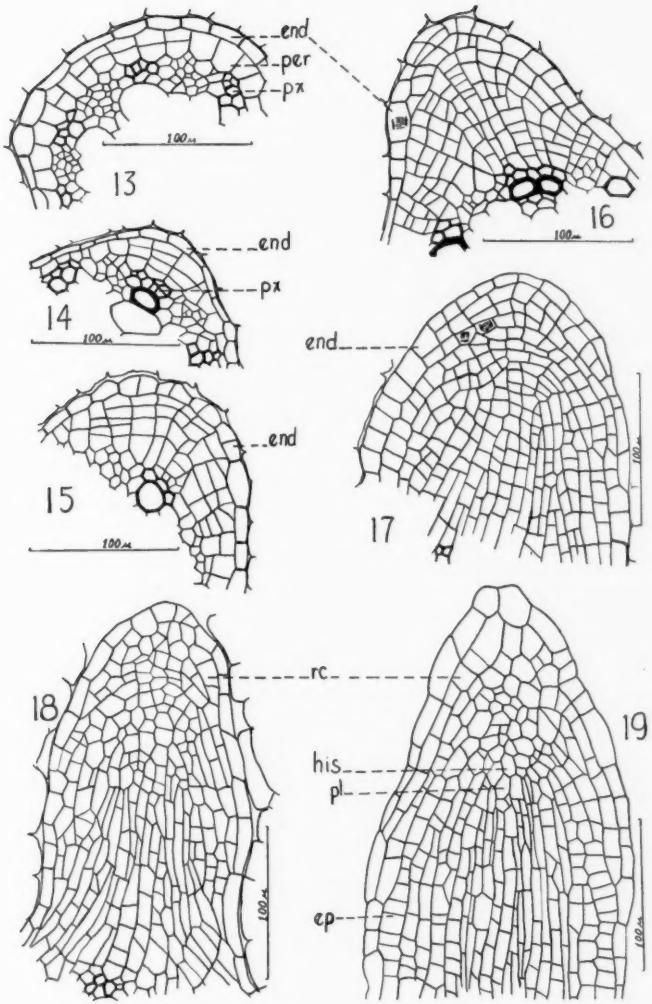
order, from the lower side of the developing rhizome with such frequency and compactness that they become very crowded as the massive crown develops (figs. 10–12). These fleshy roots, with a diameter two to six times greater than the primary root, possess a pith, a large number of alternating xylem and phloem strands, and an extensive development of cortical tissue. The outer rows of cells of the cortex become thick-walled and suberized.

Lateral roots

Lateral roots originate in the pericyclic region of the primary root before the thickening of the walls of the large metaxylem vessels occurs. These branch roots are first noted as meristematic activity of the pericyclic cells usually directly opposite the protoxylem point. The first division of the pericycle is a tangential one (fig. 13). This is followed by other tangential divisions until four to six rows of cells are formed, when radial divisions also occur (figs. 14, 15). These divisions occur somewhat irregularly, but the cells tend to be laid down in rows parallel to the radial walls of the original pericyclic cells. Continued divisions of the pericycle produce a conical mass of meristematic tissue (figs. 16, 17). A secondary root halfway through the cortex of the primary root shows differentiation into two histogenic regions as found in the primary root (figs. 18, 19).

At about the time the first tangential divisions of the pericycle occur, the endodermal cells defining the tip of the root elongate and increase in size, keeping pace with the development of the pericycle (fig. 14). When radial divisions of the pericyclic cells occur, the endodermis over the developing root tip also undergoes radial divisions (fig. 15). Radial divisions of the endodermis are followed by tangential ones (figs. 16, 17), and by this means a considerable portion of the "temporary" root cap is derived from the endodermis (figs. 18, 19).

Lateral roots extend through the cortex partly by mechanical pushing and partly by digestion of the older cortical cells. The first is evidenced by the crushed cortical cells about the young root tips. In later stages there is a lack of accumulated débris about the tip of the secondary root, which may be considered as evidence that diges-



FIGS. 13–19.—Transverse sections of portions of young primary roots showing origin of lateral roots (*px*, primary xylem; *per*, pericycle; *end*, endodermis; *ep*, epidermis; *pl*, plerome; *his*, histogen for cortex, epidermis, and root cap; *rc*, root cap).

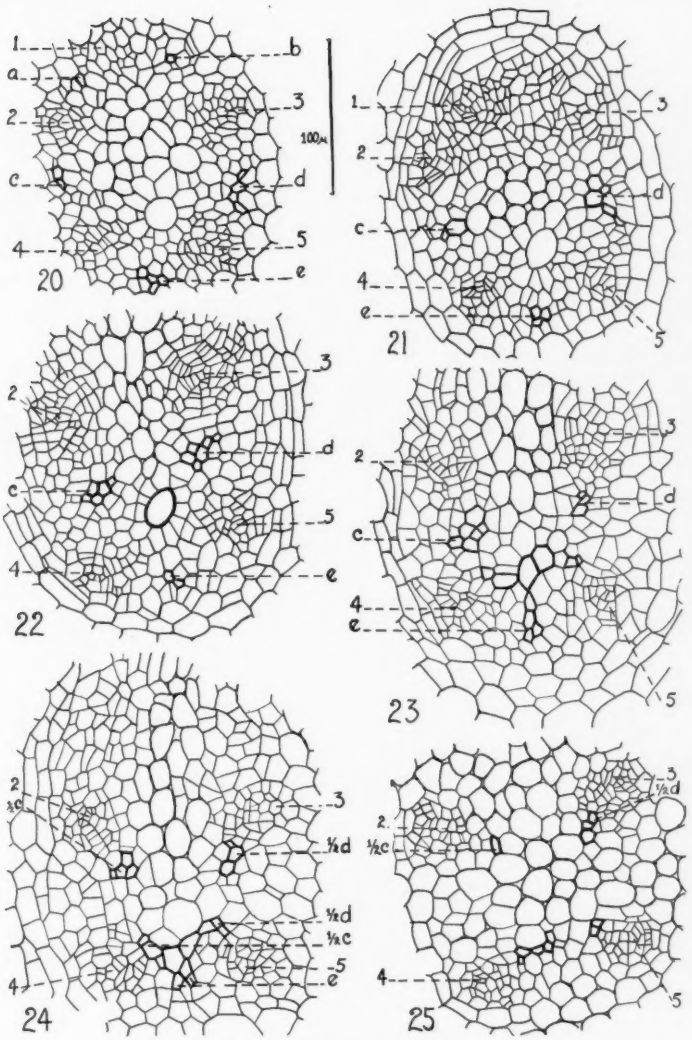
tion is important in the passage of secondary roots through the cortex.

These secondary roots continue development and extend beyond the limits of the primary roots as fibrous branches. They are usually triarch, with one large metaxylem vessel at the center.

TRANSITION REGION OF YOUNG EMBRYO

In a young embryo the cotyledon and the epicotyl are more or less in the same straight line, and in a longitudinal section the epicotyl appears as a somewhat lateral structure. Owing to the slight development of the epicotyl and the fact that very little conductive tissue is differentiated at this early stage within it, the transition must be determined largely as it takes place in the upper region of the hypocotyl and the cotyledon.

In a series of sections of a young embryo about 4 mm. long, in which the epicotyl is still only slightly developed, the transition from hypocotyl to cotyledon is evident. The orientation of the stelar tissues at successive levels from hypocotyl to cotyledon depicts the transition from the radial arrangement in the hypocotyl to the collateral arrangement in the cotyledon. A transverse section through the hypocotyl about 180 μ below the base of the epicotyl has the typical radial arrangement of the stelar structure. For convenience in following the orientation of tissues, the individual phloem and xylem strands have been given numbers and letters (fig. 20). All the xylem strands are not equally well differentiated, since the three (*c*, *d*, *e*) opposite the region which is directly below the apparently slightly lateral epicotyl are larger. A transverse section 80 μ higher includes the base of the cotyledonary sheath and the tissue just below the base of the epicotyl (fig. 21). The two weaker xylem strands (*a*, *b*) are not differentiated at this level, and xylem strands *c*, *d*, and *e* are slightly nearer the center. The phloem maintains its relative positions. At a still higher level, 100 μ above the first previous section, phloem strand *1* extends toward the base of the epicotyl. Extensions from the phloem strands *2* and *3*, located right and left of *1*, also are differentiated in the direction of the base of the epicotyl. The three stronger xylem strands (*c*, *d*, *e*) are nearer the center than in the previous section (fig. 22). A section 10 μ above the third level



FIGS. 20–25.—Series of transverse sections of stele of young embryo to show transition; phloem strands numbered *1* to *5*, xylem strands lettered *a* to *e*.

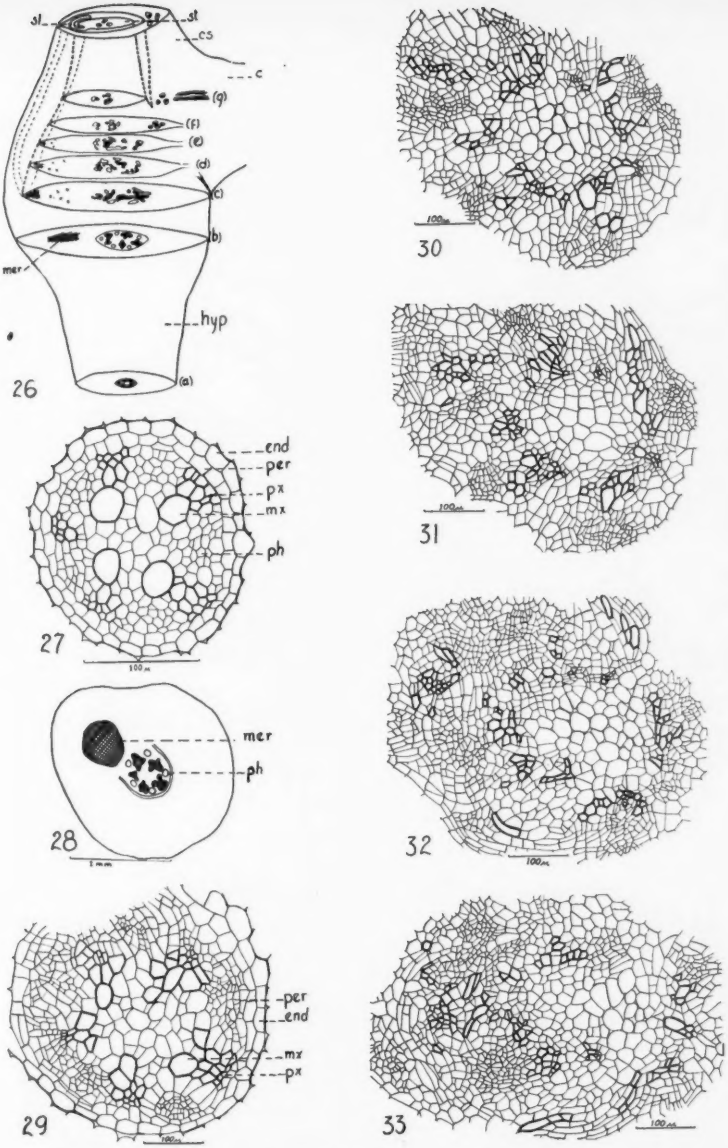
has only four phloem strands, strand *1* having ended at the base of the epicotyl (fig. 23). The xylem strands are located nearer the center than in the section previously discussed; the metaxylem appears as a broken band, owing to lateral extensions right and left, and occupies a tangential position interior to the phloem strands. The fifth section, taken 10 μ above the previous one, shows the xylem located immediately interior to the phloem strands, toward the center of the cotyledon, the portions abutting on phloem strands *2* and *3* being extensions of the halves of the adjacent xylem strands *c* and *d*. The other halves of the xylem strands *c* and *d* are found abutting on phloem strands *4* and *5*. The lateral portions of the metaxylem of xylem strand *e* are found right and left, lying against phloem strands *4* and *5* (fig. 24). In a section 30 μ above this one all the bundles are collateral, the xylem, except for a small portion of strand *e*, being located against the phloem and nearer the center of the cotyledon (fig. 25). This portion of *e* at higher levels is found as an isolated strand of xylem.

In another young embryo there are five phloem and five xylem strands in the hypocotyl and only three vascular bundles in the cotyledon. In this instance, near the base of the epicotyl, two of the hypocotyledonary phloem strands and the three xylem strands nearest these are no longer present. The remaining three phloem strands are continuations of the three collateral bundles of the cotyledon. The xylem of these cotyledonary bundles is a continuation of the two xylem strands which alternated with these phloem strands in the hypocotyl.

Transition region of seedling

A seedling 47 mm. long was used as a type for study of transition in a young plant possessing a developed stem axis. The zone of transition is short and most of it was found to occur below the point of divergence of the cotyledon from the base of the epicotyl (fig. 26). In a transverse section of the hypocotyl, a little more than 1 mm. below the divergence of the haustorial portion of the cotyledon, the exarch radial arrangement is present (fig. 27).

A transverse section through the enlarged portion of the hypocotyl, 230 μ below the divergence of the cotyledon, has a localized meristematic region in the cortex (fig. 28). This is associated with

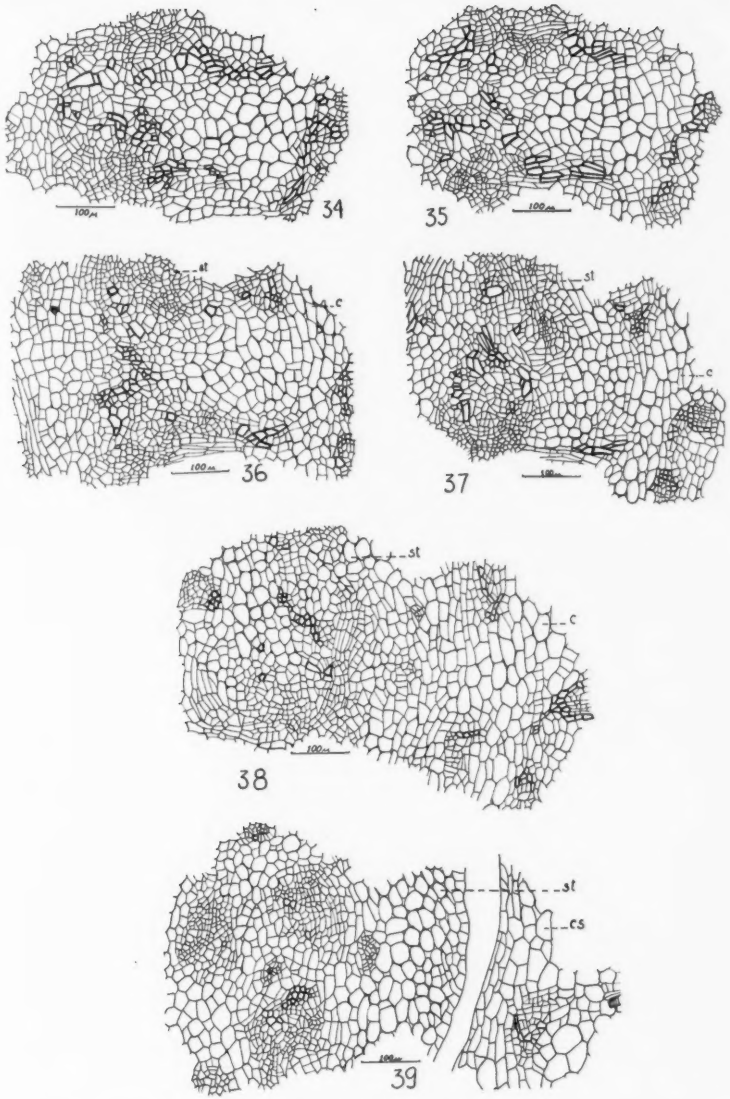


FIGS. 26–33.—Fig. 26, diagrammatic reconstruction of part of a seedling to show transition: sections at (*a*), (*b*), (*c*), (*d*), (*e*), (*f*), and (*g*) represent levels of figs. 27, 29, 30, 33, 36, 38, and 39. Fig. 27, transverse section of stelar portion of hypocotyl at level indicated by (*a*) of fig. 26. Fig. 28, diagram of transverse section of hypocotyl at level indicated by (*b*) on fig. 26. Figs. 29–33, part of a series of transverse sections, at successively higher levels, of transition region (*cs*, cotyledonary sheath; *sl*, scale leaf, *c*, cotyledon; *hyp*, hypocotyl; *st*, stem; *mer*, meristematic region).

the development of the bud which arises in the axil of the first scale leaf of the primary stem. The endodermis and pericycle are no longer evident on the side of the stele adjacent to this area, and the location of parts of the stele varies from the preceding section (fig. 29). The protoxylem points are a little nearer the center of the axis and the innermost metaxylem is situated laterally right and left and lies tangentially interior to the phloem, which maintains its relative positions.

In a section at the level of the divergence of the cotyledon there is a division of the conductive tissue into that part which is to continue into the stem and that part which is to continue into the cotyledon (fig. 30). The cotyledonary portion has four collateral bundles with some isolated xylem strands between these, the whole arranged in a circle. The cauline portion is identified by the location of some phloem and xylem outside of this circle. The arrangement is not clear, but the phloem lies external to the xylem. In the succeeding sections (figs. 31–37), 40, 110, 150, 170, 210, 270, and 310 μ above, there is considerable confusion in the arrangement of xylem and phloem tissues, since portions of many of the bundles appear in longitudinal rather than in transverse sections. This is largely due to the fact that the very short lower portion of the stem develops horizontally as a portion of the rhizome before growing upward as the aerial stem. This is not evident from the external structure because such a small portion of the stem is involved. Extensions of the vascular tissue toward the axillary bud may also account for some of the irregularity. Since the first scale leaf has no vascular tissue, it does not influence this confusion of tissues.

The haustorial portion of the cotyledon contains four vascular bundles. The cotyledonary sheath extending upward contains only three. One of these is larger and represents the upper undiverged portion of two bundles. The bundles of the haustorial portion connect at right angles with the bundles extending vertically from the cotyledonary sheath into the hypocotyl. The isolated xylem strands present in the lower portion of the stem axis (figs. 38, 39) apparently have no significance, since at higher levels they are identified as parts of the xylem of the nearest bundles. This also obtains in the cotyle-



FIGS. 34–39.—Continuation of series of transverse sections at successively higher levels of transition region of a seedling.

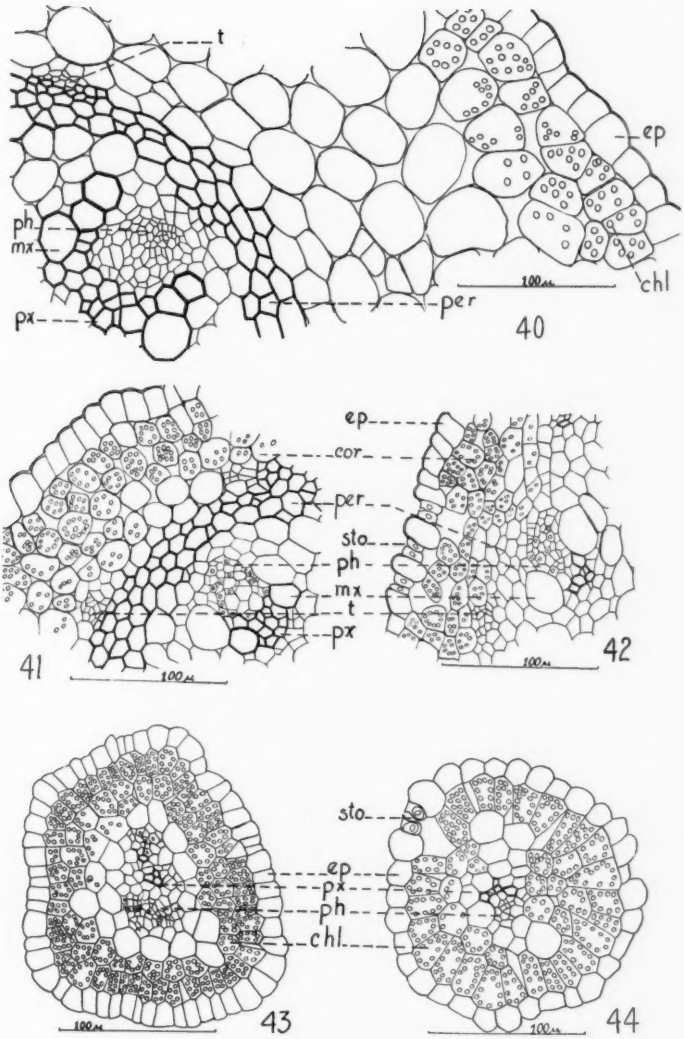
dons where isolated xylem strands are found to be portions of the collateral bundles.

Other seedlings of similar age were examined for transition and were found to be similar to the one described. The transition may be considered a modification of type A of EAMES and MACDANIELS (7) or of type I of VAN TIEGHEM (15).

STEM

The lower portion of the main axis grows out horizontally and forms a small portion of the rhizome before the distal portion extends upward as an aerial shoot. The primary stem is slender and usually less than 2 dm. in height. The lower scale leaves (the first, second, and third) usually contain only branches in their axils. The upper scale leaves may bear both branches and cladophylls (fig. 10).

A transverse section of the primary stem reveals three zones. The first is the cutinized epidermis with stomata. The second is a varying number of rows of large cortical cells, the outer ones photosynthetic and with large intercellular spaces. The third is the stele, which may be divided into three parts: (a) a few rows of pericyclic cells which are strongly lignified in the lower, older portions of the stem and (b) the ground parenchyma located in the center between (c) the irregularly arranged vascular bundles (figs. 40–42). The larger bundles located near the center are the leaf traces, one large bundle associated with each leaf. These bundles are larger and more nearly centrally located in the vicinity of the node where they are diverged from the stele. At lower levels of the stem they are found near the periphery of the central parenchyma, in which region they may end blindly although usually they anastomose with traces of leaves at lower nodes. At the base of the stem there are anastomoses between the bundles of the stem and those of the rhizome. The small bundles which are found peripheral to the pericycle are portions of branch traces. In the upper, younger portions of the primary stem only one new branch trace is found at a node. At lower levels a second trace is differentiated and is present in the main stem at the node. Many anastomoses occur with the common bundles of the stem, but small portions of these branch traces, which do not immediately become part of the central stele, are found in the pericycle



FIGS. 40–44.—Figs. 40–42, parts of transverse sections of lower, middle, and upper portions of primary stem. Figs. 43 and 44, transverse sections of cladophylls (*px*, primary xylem; *mx*, metaxylem; *per*, pericycle; *cor*, cortex; *ep*, epidermis; *sto*, stoma; *t* branch trace; *chl*, photosynthetic area).

through an extent of several internodes before they pass through it and are found in the periphery of the central parenchyma. Here two or three may converge and anastomose with other traces.

The vascular bundles possess a V-shaped xylem arrangement with large reticulated vessels at the apices of the arms of the V and smaller spiral protoxylem vessels directed toward the center of the axis. The arms of the V inclose the phloem.

The bud, arising in the axil of the first scale leaf of the primary shoot, gives rise to a branch the short proximal portion of which forms the continuation of the rhizome, the distal portion the aerial shoot. The bud arising in the axil of the first scale leaf of this second shoot gives rise to the third shoot. As this process is continued, the bases of the aerial shoots form a compact rhizome made up of short internodes. Since the succeeding buds arise alternately right and left, the rhizome has two rows of spears in a close zigzag arrangement. Occasionally accessory buds develop, forming branches of the rhizome.

At the end of the growing season the aerial stems die, leaving scars on the older part of the rhizome. At the tip of the rhizome, however, buds for aerial stems of the next season are already present.

CLADOPHYLLS

The cladophylls are essentially stem structures (fig. 10). They are terete or polygonal in transverse section and have no morphological dorsiventrality. There is a cuticularized epidermis with slightly depressed stomata, the guard cells of which are considerably smaller than the vertically elongated epidermal cells. This differs from ZWEIGELT'S description (**17**) which includes accessory cells associated with the stomatal apparatus. Beneath the epidermis there are two to three rows of palisade cells crowded with chloroplasts. These are followed by the ground parenchyma and from one to four collateral vascular bundles. Two was found as the common number in young cladophylls (fig. 43). A section near the apex of one has a single vascular bundle (fig. 44). In older cladophylls lignified sheaths are developed about the vascular bundles. The vascular connection of the cladophylls with the primary stem is made through an anastomosis of the small bundles of the cladophylls with the large bundle of the scale leaf.

Scale leaves

The sessile scale leaves are approximately triangular in shape, but possess a small, median, ventral prolongation. Usually no vascular tissue or very little is present in the first scale leaf. In the others there are usually four main bundles, two of which extend laterally at the base, and two of unequal size which extend upward in the central region. Small vascular bundles may extend upward from the two lateral bundles. All of these bundles converge into one at the base of the leaf, and vascular connection with the stem is made through this bundle.

Summary

1. The structure of the embryo and the development of the seedling of *Asparagus officinalis* L. are described.

2. The primary root is usually pentarch or hexarch. In its growing point there are two histogens, the inner giving rise to the stele and the outer to the cortex, epidermis, and root cap.

3. Lateral roots are initiated by tangential divisions of cells of the pericycle. Subsequent tangential and radial divisions continue the development.

4. In the development of lateral roots the endodermis becomes active and divides by tangential and radial divisions to form a considerable portion of the "temporary" root cap.

5. Transition from an exarch to an endarch condition of the xylem occurs in the hypocotyl and the bases of the cotyledon and the epicotyl. The transition is complicated by the development of both a part of the rhizome and the first aerial branch from the epicotyl.

6. The rhizome is formed from horizontal outgrowths of the base of the epicotyl and the bases of successive aerial stems.

7. Successive aerial branches arise from buds in the axils of the first scale leaves of the preceding aerial branches.

8. The leaves are scale leaves with one large trace which extends toward the center of the central parenchyma of the stem. From this region the trace gradually diverges outward until, at lower levels of the stem, it is found near the periphery of the central parenchyma. Here it may end blindly but usually anastomoses with traces of leaves of lower nodes.

9. The young branch, arising in the axil of a scale leaf, has small

traces which anastomose with the common bundles of the stem. Small branches of these traces remain in the outer pericycle, through several internodes of the main stem, before they pass through it and come to lie in the peripheral region of the central parenchyma, where they anastomose with other bundles.

10. The cladophylls are essentially stem structures. They arise in clusters in the axils of the scale leaves.

The writer gratefully acknowledges the helpful suggestions and criticisms of members of the Department of Botany of the University of Chicago during the progress of this investigation.

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A METHOD OF MEASURING RESPIRATION AND CARBON FIXATION OF PLANTS UNDER CONTROLLED ENVIRONMENTAL CONDITIONS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 467

JOHN W. MITCHELL

(WITH FIVE FIGURES)

Introduction

In various studies of the rates of respiration and carbon fixation, many methods have been developed to control as nearly as possible the several environmental factors involved. As yet none has proved satisfactory. It is the purpose to give here a statement of the methods and a brief description of the apparatus used in making such studies when the plants used were inclosed in glass chambers and exposed to a moving air stream. Temperature, light, humidity, CO_2 supply, and the rate of air circulation were controlled in such a way as to maintain nearly constant conditions inside such chambers.

Temperature was readily controlled by means of an insulated room having properly regulated thermostats, cooling system, and heaters. A means of controlling the humidity inside the chambers was not necessary since it remained nearly constant during the period over which measurements were taken. Because of the wide fluctuations in intensity and quality of natural daylight, it was necessary to employ some other source of illumination which could be precisely regulated. An arc lamp was finally selected as the most suitable source of artificial illumination. It was found desirable to enrich the air stream supplied the plants under test by the addition of a known amount of CO_2. The air around the leaves under study constantly recirculated. This was necessary to make sure that the leaves or plants were constantly bathed by a more nearly uniform concentration of CO_2 and also to avoid the use of quantities of gas supplied in excess of the amount that could be readily and accurately measured.

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

Details of apparatus used

TEMPERATURE.—An insulated room, provided with coils connected to an ammonia compressor and temperature control unit, was used. By means of this apparatus it was possible to maintain a room temperature of 69°–71° F. during periods of illumination, and 64°–66° F. during periods of darkness.

When the light was on, during the measurements of rates of carbon fixation, the temperature of the air inside the leaf chamber rose about 4° C. above that of the room. In order to measure the temperature of the leaves themselves, a potentiometer and thermocouples made from fine constantine and copper wire, insolated with a thin coating of paraffin, were employed. Readings were made with the couples against the lower side of the leaves as well as threaded through them. The readings were approximately the same regardless of the position of the couples. During illumination the temperature of the leaves increased approximately 2° C. above the surrounding air, whether inclosed in the chambers or not. MILLER (3) found similar differences between the temperature of leaves and the surrounding air under natural conditions.

HUMIDITY.—The relative humidity of the air in the room ranged between 60 and 75 per cent, while that of the air inside the chambers ranged from 50 to 80 per cent, depending upon the size and type of leaf used. When large succulent leaves were used, the relative humidity in the chamber usually ranged from 65 to 70 per cent and when small leaves were inclosed the range was from 50 to 65 per cent.

ILLUMINATION.—In the selection of a source of artificial light under which various plants, particularly the tomato, could be suitably grown, several qualities and intensities of light were tested. Tungsten filament electric lamps varying in output from 500 to 2000 watts were first tried. The quality of light from such a source was altered by using gelatin filters of various types, and also by operating 105, 108, and 110 volt lamps at 112 volts. When supplemented by no other source of light, plants which had previously been grown to some size before being placed under experimental conditions showed some variation in their color but none of them accumulated an

appreciable carbohydrate reserve, and all showed symptoms of etiolation within seven days. The use of a considerable number of such lamps in a room means the production of a great quantity of heat. As a result it was not possible under local conditions to control room temperatures readily. The use of tungsten lamps then was abandoned and an Eveready carbon arc lamp substituted. The arc of this lamp was completely inclosed by glass filters, held in place by removable metal frames. By means of an exhaust fan a constant supply of fresh air was drawn from outside the experimental room to the bottom of the glass inclosed chamber about the arc, through this chamber, out at its top, and exhausted outside the building. In this way it was possible to remove most of the heat and any gases which might influence the development of the plants.

This arc lamp operated on a current of 60 amperes with 50 volts at the arc. Plants were placed in a circle around the lamp at a distance of 30 inches, the light striking the leaves at an angle of 45°. Carbons known as the "sunshine" type produced a suitable quality of light. Energy radiation from the arc produced by such carbons consisted qualitatively of a spectrum which was practically as continuous as that of sunlight. A spectrogram shows that the spectral lines extend from the infrared through the visible and ultraviolet to approximately 2800 A.u. A comparison of the energy radiation curves of noon-day summer sunlight and artificial light from sunshine carbons (fig. 1) shows that sunlight contains more red, but less blue. The quality of natural sunlight itself is by no means constant and measurements vary greatly, depending upon the time and place at which they are made. On the basis of an assumed average quality of natural sunlight, the quality of light produced by sunshine carbons gives a closer approximation than does the light produced by any of the other types of carbons or by the tungsten filament lamps. To be of use in the present experiments, however, it was found that a filter was necessary to remove the excess amount of ultraviolet. With a filter of Corex D glass, which transmits most wave lengths from the visible down to about 2500 A.u., tomato and bean plants were killed upon being exposed to this light for a period of 2–5 hours. The use of pyrex glass filters, which removed rays shorter than 2700 A.u., resulted in injury to plants in 1–2 weeks. Single strength window

glass, which removed most rays shorter than 3200 A.u., was finally selected, since no injury resulted from its use.

As previously indicated, it is difficult to make a quantitative comparison of artificial light and natural sunshine, owing to the great range of variability in the intensity of natural light. A rough comparison of intensities of the light from screened arc and natural daylight on cloudy and clear days was made. Measurements made with

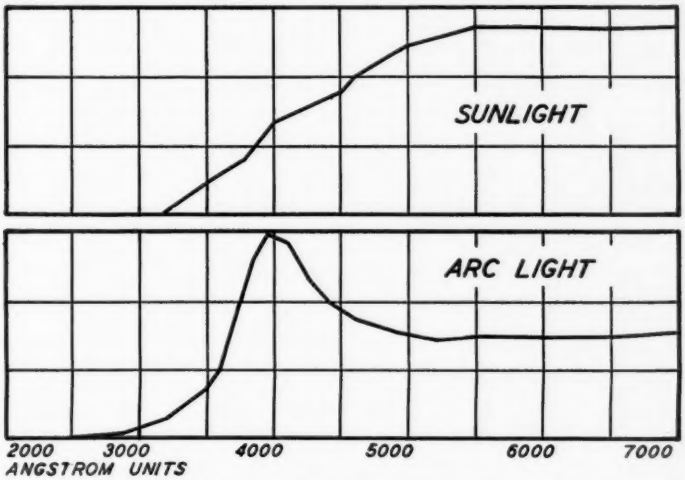


FIG. 1.—Comparison of energy radiation of noon-day summer sunlight and arc light from "sunshine" carbons. In the graph illustrating sunlight each square represents 2500 micro-watts of radiant flux per square cm. Each square in the graph illustrating arc light represents 250 micro-watts per square cm. at 1 meter distance from the arc. By courtesy of the National Carbon Company.

a thermopyle, MacBeth illuminometer, and Weston light meter showed that the intensity of total or visible radiant energy of the arc at a distance of 3 feet was very much less than that of direct sunlight at noon on a clear day in April. However, the intensity of total or visible radiant energy from the arc light exceeded that of natural light during a day in the same month when the sky was completely overcast. In some of the first experiments performed, it was noticed that there was a 15–20 per cent increase in the total radiant energy produced by any given set of carbons during the last half of their

burning time. This increase in intensity was possibly due to the infrared radiations produced by metallic parts of the lamp which moved nearer to the arc and increased in temperature as the carbons were consumed. Such an increase in intensity was entirely eliminated by passing the light from the arc through a water filter. The continued use of such a water filter was not necessary, however, as experiments have shown that a 20 per cent increase in intensity of infrared radiation, during the life of a set of carbons, has no noticeable effect on the rate of carbon fixation by the plants studied.

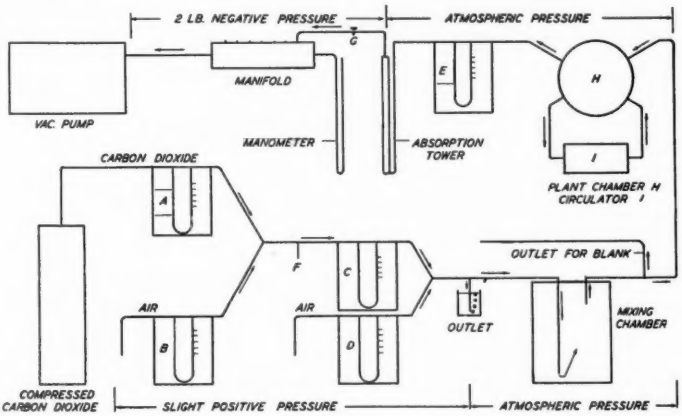


FIG. 2.—Arrangement of apparatus used to measure rates of respiration and carbon fixation.

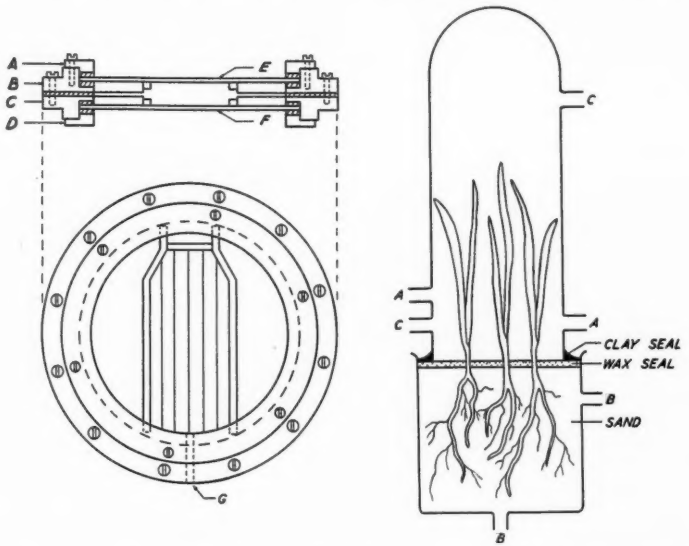
MOVEMENT OF ENRICHED AIR THROUGH THE SYSTEM.—The volume of gas which was supplied to the leaf chambers was derived from a tank of compressed air, the volume available at any particular time being slightly in excess of the amount subsequently to be drawn over the leaves. A flowmeter (fig. 2*D*) of the type described by WILSON and GEORGI (7) was used to indicate the flow. The CO_2 which was used to enrich the main air stream was derived from tanks of the compressed gas. From such a tank the gas was first fed through a flowmeter (fig. 2*A*) and mixed with air coming from the compressed air tank at a known rate as indicated by the flowmeter (fig. 2*B*). In this manner suitable quantities of a mixture of air and CO_2 in any

desired proportions could be had. In practice the volume available was always slightly in excess of the amount finally to be drawn over the leaves in the chambers. Such an excess was allowed to escape through an outlet immersed in oil, thus preventing any other gases from entering the system. Up to this point the gases were under slight positive pressure. From now on, however, the gases traveled through the remainder of the system because of a negative pressure set up by the use of a vacuum pump at the extreme end of the line, the degree of negative pressure at any point in the system being regulated by needle valves and determined by flowmeters at various points as described later. Thus beyond the escape valve just mentioned the air enriched with CO_2 passed, at virtually atmospheric pressure, through copper tubing to the leaf chamber, where it was recirculated several times by means of a special pump connected with the chamber, before passing on out of the chamber into a tube which fed through a flowmeter to an absorption tower, kept at constant temperature by immersion in a controlled water bath. The rate of flow into the tower was controlled by a needle valve (fig. 2*G*) and the flow gauged by a meter (fig. 2*E*). After passage through the absorption tower, the remaining gases passed through copper tubing into a manifold where a slightly greater negative pressure was maintained and controlled by a needle valve and indicated by a manometer. Beyond the needle valve a tube led directly to the vacuum pump.

Seven sets of the apparatus described were assembled as a unit and operated by a single vacuum pump. Thus as many as seven air streams could be drawn from a common source simultaneously, six of them across leaves or plants, the seventh used as a check to determine the amount of CO_2 in the enriched air supply.

LEAF AND PLANT CHAMBERS.—Two types of chambers were devised, one of which was used to inclose an attached leaf, the other being used to inclose an entire plant. The frame of the first type was constructed from four brass rings of the relative dimensions shown in figure 3 (*A*, *B*, *C*, *D*). Corex D glass was used in the side of the chamber nearest the light source (fig. 3*E*), while single strength window glass was used in the side away from the light (*F*). Rubber gaskets were inserted on each side of both glasses and shellac applied

to prevent air leakage. A removable metal frame bearing fine wires was inserted in each half of the chamber for the purpose of holding the leaf away from the glass. The excess space outside of these frames was filled with plaster of Paris to decrease the volume within the chamber. Several applications of a commercial lacquer were used to reduce the porosity of the plaster and thus prevent the adsorption



FIGS. 3, 4.—Fig. 3 (left), diagram of leaf chamber. Fig. 4 (right), diagram of plant chamber; air stream connected to outlets *AA*, another to *BB*, and a circulator to outlets *CC*.

of appreciable quantities of gas. The parts of the chamber were fastened together with screws and the union between the upper and lower halves made air tight by means of a rubber gasket. A hole was drilled at the union of the two halves of the chamber through which a leaf petiole could be inserted (fig. 3*G*). Four pieces of pipe, extending through the metal walls, made it possible to recirculate air through the chamber. The chamber was finally mounted on an adjustable stand as shown in figure 5*A*.

The second type of chamber consisted of an upper part inclosing

the stems and leaves and a lower part inclosing the roots of the plants (fig. 4). The lower part of the chamber consisted of a 1.5 liter beaker provided with two outlets through which air could be drawn. One of these outlets was at about the center of the bottom, and the other about three-fourths of the distance up the side. The beaker was filled with quartz sand in which plants were grown until they attained the desired height. A paraffin, vaseline, and mineral oil

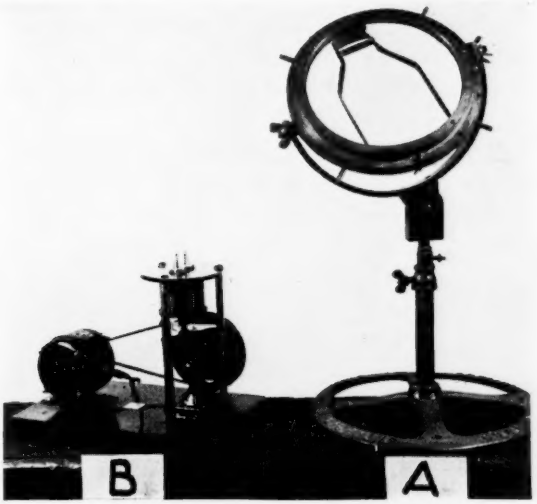


FIG. 5.—*B*, circulatory pump; *A*, leaf chamber

mixture, having a melting point of 38° C., was heated to a temperature of 41° C., then poured over the surface of the sand and allowed to flow around the stems of the plants and solidify. Thus the lower portion of the chamber was effectively sealed, so that gases could neither enter nor leave except through the openings already mentioned.

The upper part of the chamber, which was constructed of large pyrex tubing and provided with four openings, was placed over the portion of the plants extending above the paraffin seal. An air tight connection was made between the upper chamber and the paraffin seal by means of a mixture of clay and castor oil.

Through two of the openings gas entered and left the chamber at the rate of 15 liters an hour. This rate of movement over the leaf surface, however, was not rapid enough for the most effective absorption of CO_2. Consequently tubes leading to a circulation pump were attached to the other two openings so that gases in the chamber were constantly agitated and moved over the leaf surfaces at a rate equivalent to a complete flushing of the chambers 200 times per hour, or a volume of gas equivalent to 400 liters.

CIRCULATORY PUMPS.—A pump was devised to circulate the air inside the chamber. Its usefulness depended on the fact that it could be made air tight and could circulate the desired volume of air for a period of several weeks without attention.

In the construction of these pumps a length of metal bellows 3 inches in diameter and 2.5 inches long was attached to a circular brass plate held in a horizontal position by upright rods (fig. 5*B*). Inlet and outlet valves were inserted in the plate. Another circular brass plate, somewhat smaller in diameter, was attached to the lower end of the bellows and connected to an excenter on a drive shaft by means of a connecting rod and suitable bearing. The mechanism was operated by a small motor.

A pump of this type delivered from 350 to 500 liters of air per hour. When attached to a leaf chamber having a volume of 500 cc., the volume of gas in the chamber was flushed at the rate of approximately 800 times per hour. The minimum volume of gas circulated over a leaf having an area of 100 square cm. was therefore between 30 and 40 liters per square cm. of leaf surface per hour. This rate of circulation was greatly in excess of that required for the maximum rate of carbon fixation by leaves, as shown by the work of HEINICKE and HOFFMAN (**2**).

ESTIMATION OF CO_2.—The conductivity method was used for the estimation of CO_2 in an air stream to which leaves had been exposed. The absorption towers were similar in construction to those described by THOMAS (**6**).

These towers were charged with 50 cc. of LiOH solution containing 0.5 to 1.0 per cent of butyl alcohol. Air moving at a rate of 15 liters per hour through the absorbent was dispersed as fine bubbles not more than 2 mm. in diameter.

The procedure used in the standardization of an absorption tower and alkali absorbent solution was as follows: the tower was filled with 50 cc. of absorbent, placed in a water bath, and brought to the temperature to be used. It was then connected to an air stream and an amount of CO_2 was collected. The electrical resistance of the solution was then determined with a wheatstone bridge. The solution was removed from the tower and an aliquot titrated with HCl as described by FRIEDEMANN and KENDALL (1). This process was repeated several times, absorbing larger amounts of CO_2 for each determination. A curve was finally drawn from the data collected, which indicated the resistance of the alkali solution in the tower used in terms of milligrams of CO_2. Towers and solutions standardized in this way have been used over an extended period without a noticeable change after standardization.

Dependability of results

It has been shown by SPOEHR and MCGEE (5) and NEWTON (4) that very small amounts of CO_2 may be accurately measured by the conductivity method. It should be noted, however, that the efficiency of dilute alkaline solutions used to absorb CO_2 decreases as the hydroxide is changed to bicarbonate and carbonate, through the addition of CO_2. As much as 150 mg. of CO_2 could be absorbed by 50 cc. of 0.25 N LiOH under the conditions used, without appreciably limiting the absorbing capacity of the alkali.

In the use of an apparatus which measures simultaneously the rates of respiration or carbon fixation of several leaves, it is important to know that the same degree of accuracy is exhibited by the several separate units of the system. Two air streams, drawn simultaneously from a common air source, were analyzed to determine the accuracy of the method described in this study. The movement of air in the two lines was adjusted to an equal velocity. A total of 95 liters of air was drawn through each system and the amount of CO_2 collected from each air stream was determined at the end of every period required for the passage of 19 liters. The data thus obtained showed the difference between the amounts of CO_2 collected from the two air streams to be less than 5 per cent. Analyses were also made, using six separate units of the system simulta-

neously, the results showing that 0.61, 0.59, 0.58, 0.59, 0.58, and 0.61 mg. of CO_2 per liter were collected from air drawn through the six respective units. Again an error of about 5 per cent resulted.

Tomato, bean, primrose, geranium, and cineraria plants produce healthy vegetative growth under the environmental conditions described. A comparison of the approximate rates of CO_2 assimilation

TABLE I

COMPARISON OF APPROXIMATE RATES OF CARBON FIXATION BY VARIOUS PLANTS. THE CONCENTRATION OF CO_2 IS THAT WITHIN THE RANGE OF VARIABILITY FOUND UNDER NATURAL CONDITIONS

DATE	PLANT	UPTAKE MG. CO_2 PER HOUR PER 100 SQ. CM. LEAF SURFACE	LIGHT SOURCE	INVESTIGATOR
1884.....	Sunflower	18.0	Sunlight	Sachs
1917.....	Pumpkin	18.0	Sunlight	Miller
1917.....	Cow pea	18.5	Sunlight	Miller
1917.....	Soy bean	8.0	Sunlight	Miller
1920.....	Sugar cane	3.5	Sunlight	McLean
1933.....	Apple	15.0	Sunlight	Heinicke and Hoffman
1934.....	Geranium	16.5	Arc light	Mitchell
1934.....	Tomato	17.0	Arc light	Mitchell
1934.....	Primrose	13.5	Arc light	Mitchell
1934.....	Cineraria	13.5	Arc light	Mitchell
1934.....	Wax bean	13.0	Arc light	Mitchell

by leaves of these plants under the conditions described and the results obtained by other investigators using other methods is given in table I.

Summary

1. An apparatus is described with which the hourly rates of carbon fixation or respiration of attached leaves can be accurately determined. Descriptions of a new type of circulatory pump and two new types of leaf chamber are given.

2. Artificially controlled environmental conditions are described in which several species of plants have gained in dry weight over a period up to four weeks.

3. Of the sources tested, an Eveready carbon arc lamp, inclosed by a housing of window glass, was found to be the most suitable for maintaining plants under conditions of artificial light.

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CORRELATION OF SHAPE OF FRUITS, COTYLEDONS, AND SEEDS IN MELONS[1]

LESLIE M. WEETMAN

(WITH EIGHT FIGURES)

In connection with the breeding of wilt-resistant watermelons (*Citrullus vulgaris* Schrad.), it was observed that plants with ovate or nearly circular cotyledons commonly bore spherical fruits and that plants with elongated cotyledons bore elongated fruits. This was particularly noticeable in the new wilt-resistant Iowa Belle variety, which originated from a chance hybrid heterozygous for fruit shape. The apparent correlation between shape of cotyledons and shape of fruits suggested that the shape of the fruits a plant will bear might be foretold by observing the shape of the cotyledons. This would greatly expedite the selection of strains homozygous for round or long fruits. With this idea in view, a study was started to determine more definitely the relationship between shape of cotyledons and shape of fruits in the Iowa Belle and in a number of other varieties. It was also desirable to determine whether there is a correlation in shape between seeds and cotyledons and between seeds and fruits.

HUTCHINS (6) has reported that the cotyledons, fruits, and seeds of cucumbers are closely correlated in shape. He calculated correlation coefficients from the mean shape indices of a number of varieties and from individual indices in an F_2 population of 300 plants. Length and shape of cotyledons and seeds were more influenced by the female than by the male parent in hybrids. With regard to matrocliny in the cotyledons of hybrids, FOCKE (2) cited the observations of CASPARY that reciprocal hybrids of *Nymphaea rubra* and *N. dentata* differed in the shape of the cotyledons. The cotyledons of the hybrid resembled those of the female parent in each case. GROTH (4) found in tomatoes that both size and shape of F_1 cotyledons were affected by the size and possibly by the shape of the seed

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of the female parent. HILL (5) reported that reciprocal F_1 hybrids of species of *Digitalis* differed considerably in the form and size of their cotyledons, and that they always resembled the female or seed parent in these respects, although this matrocliny was not complete. He thought the explanation lay in the influence of the maternal seed coats upon the developing embryos. LUBBOCK (7) described seedlings of a number of species of the Cucurbitaceae and attributed the shape of the cotyledons in part to the space they occupied in the seeds. GOEBEL (3), however, considered that the form of the seed did not necessarily determine the configuration of the cotyledon. THAYER (9) found in hybrids of *Cucurbita pepo* a high correlation of shape of cotyledons with shape of the seeds from which the cotyledons came, although there was evidence of some normal biparental inheritance also.

Material and methods

Coefficients to express the correlation between shape of cotyledons and shape of fruits were calculated from shape indices. These indices were determined from measurements of cotyledons and fruits. The formula used to find the index numbers of cotyledons was:

$$\text{shape index} = \frac{\text{maximum width}}{\text{maximum length}} \times 1000$$

and that for fruits:

$$\text{shape index} = \frac{\text{greatest equatorial diameter}}{\text{polar diameter}} \times 1000$$

Thus the shape index is 1000 for a spherical fruit, less than 1000 for an elongated fruit, and greater for an oblate fruit. Cotyledons in the types studied always had shape indices less than 1000.

During the summer of 1931 measurements of cotyledons and fruits were obtained for 1169 plants comprising 242 varieties and selections of watermelons and citrons. Besides these, a large number of cotyledons and fruits of 23 selections of Iowa Belle were measured without keeping records of individual plants. The plants were grown in the melon-growing district of Muscatine County, Iowa. The measurements of the cotyledons were made after the first

and second true leaves had formed on most of the seedlings (15 to 22 days after planting), the measurement of one cotyledon of each plant being considered sufficient. Usually only the largest and most perfect fruit from each vine was measured, although in some cases several fruits were used and the results averaged. Measurements were made on the cut surfaces of melons split longitudinally.

Seeds to be measured were selected at random from the packets from which the seeds planted in the field were taken. The index for seed shape was calculated in the same manner as that for shape of cotyledons.

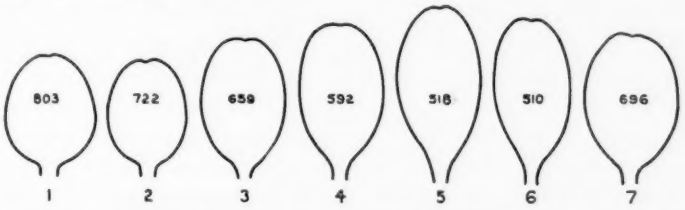
Coefficients of correlation were computed according to the methods outlined by WALLACE and SNEDECOR (**10**), and the covariance was analyzed by the method given by SNEDECOR (**8**). For each group of melons three coefficients were computed for the correlation between shape indices of cotyledons and fruits: (1) that for the *total* correlation in individual plants, disregarding varieties; (2) that for the correlation *between* varieties, the correlation in individuals within varieties being eliminated; and (3) that for the correlation *within* varieties after the correlation between varieties had been eliminated. Coefficients were tested for significance by referring to the tables of WALLACE and SNEDECOR.

Investigation

SHAPES OF COTYLEDONS AND FRUITS

There are numerous intergrading shapes in cotyledons of watermelons, the chief variation being in the ratio of maximum width to maximum length. The range in form is from short oval or slightly ovate to long elliptical (figs. 1–8). The indices of the more noticeably oval forms vary from about 690 to 750 or higher, while those of the long types range from 625 to 550 or lower. The mean shape index of the cotyledons of 1077 seedlings of 243 varieties and selections was 655. In the Iowa Belle variety the blade of the oval type of cotyledon is more or less rounded at the base, whereas the elongated type has a tapering or wedge-shaped base (figs. 1–5, 8). In very young Iowa Belle seedlings, oval cotyledons have a rugose or crimped appearance which is lacking in the elongated ones (fig. 8). This distinction disappears as the plants get older.

The variations in shape of watermelon fruits are more extreme than those of the cotyledons. In several varieties the melons are almost spherical or slightly oblate; that is, with shape indices of 1000 or more. On the other hand, certain long-fruited varieties have shape indices below 500.



FIGS. 1–7.—Outlines of watermelon cotyledons of different shapes with the shape index of each: figs. 1–5, Iowa Belle; fig. 6, Sugar Stick; fig. 7, White Seeded Chilian.

FIG. 8.—Watermelon seedlings showing shape and texture of the cotyledons: left, Kleckley Sweet with long type of cotyledons; right, Round Iowa Belle.

CHANGES IN SHAPE OF COTYLEDONS WITH DEVELOPMENT

In order to ascertain whether or not the cotyledons change in shape as they develop, measurements were taken at two different ages on a number of plants in the greenhouse. The first reading was taken when the plants were at about the stage shown in figure 8,

before true leaves had developed. The second measurements were taken 12 days later at which time the second true leaf was in evidence on most seedlings. A total of 189 plants was used in the first reading and 192 in the second, the increase in number being due to the fact that a few plants were too young to be measured at the first reading. These plants represented 24 different varieties. The mean shape index of the cotyledons was 674 at the time of the first measurements, but this mean had changed to 654 at the time of the second reading, there being a difference of 20. FISHER'S (1) method of testing the difference between two means shows this difference to be highly significant, the value of t being 3.78 whereas a t of only 2.59 would have given a probability of 0.01. The samples used were too small to test the significance of the change in each variety, although in every variety but one the mean shape index became smaller. In the one exception the index remained the same. These data indicate that in general the cotyledons of watermelons change in shape as they develop, the increase in length being comparatively greater than the increase in width. This study was not carried far enough to determine at what age the cotyledons assume a constant shape. It is interesting to compare these results with those of HILL (5), who found that in the cotyledons of *Digitalis* the ratio of width to length increased with age instead of decreasing.

CORRELATION OF SHAPE OF COTYLEDONS WITH SHAPE OF FRUITS

CORRELATION IN WATERMELONS.—The data showing correlation of shape of cotyledons with that of fruits in different groups of melons are summarized in table I. As was mentioned earlier in this paper, the methods of analysis of covariance were employed so as to obtain correlation coefficients, not only for the *total* correlation of the individuals of a group but also for the correlation *between* varieties and for that *within* varieties. In all groups of watermelons the coefficients for the total correlation of indices of cotyledon shape and fruit shape were highly significant except in group 6, which contained only 30 plants. The coefficients for the correlation between varieties were much higher than those for total correlation in all groups. The correlation within varieties was much lower than the total but was significant in all watermelons except groups 6 and 7.

Group 1 consisted of 81 inbred selections of the Iowa Belle variety. Some of these selections were homozygous for spherical fruits, some homozygous for elongate fruits, and some were heterozygous for fruit shape. Individual records were kept on 360 plants. These included

TABLE I

CORRELATION BETWEEN SHAPE OF COTYLEDONS AND SHAPE OF FRUITS IN DIFFERENT GROUPS OF MELONS

Group	Description	Number of plants	Varieties and selections	Mean shape index		Correlation coefficients		
				Cotyledons	Fruits	Total	Between varieties	Within varieties
1...	Inbred Iowa Belle	360	81	648±2	763± 6	0.72†	0.88†	0.21†
2...	Inbred Iowa Belle	.....	23	650±7*	722±30*	.....	0.96†	.....
3...	American varieties	164	28	664±3	763±10	0.58†	0.72†	0.29†
4...	Foreign varieties (includes 10 American varieties)	398	89	663±2	817± 6	0.48†	0.60†	0.16†
5...	American and foreign (groups 3 and 4)	562	117	663±1	801± 5	0.51†	0.63†	0.20†
6...	Other inbreds	30	6	637±6	713±28	0.40‡	0.66	0.07
7...	F_1 hybrids	82	14	652±5	670±13	0.79†	0.95†	0.03
8...	F_2 hybrids	43	5	621±5	602±15	0.70†	0.99†	0.38‡
9...	All watermelons (groups 1, 3, 4, 6, 7, 8)	1077	223	655±1	768± 4	0.61†	0.75†	0.20†
10...	Citrons	59	13	638±3	870±20	0.00	0.04	−0.07
11...	Watermelon-citron hybrids	33	6	651±6	603±17	0.14	0.49	−0.09
12...	*Cucumis melo*	55	19	513±4	802±15	0.27‡	0.31	0.17

* The mean of the varietal means.

† Highly significant, that is, with probability of 0.01 or less.

‡ Statistically significant, with probability between 0.05 and 0.01.

almost every variation in fruit shape as well as in cotyledon shape. Group 2 consisted of 23 of the best selections of the Iowa Belle which were grown in large numbers for breeding purposes. These were mostly homozygous for round or long fruit shape. Measurements were taken on a number of plants in each selection without keeping records of individuals. From these, the mean shape indices of the cotyledons and of the fruits were calculated for the different selec-

tions. The correlation coefficient calculated from these means was 0.96, which was highly significant as judged by the statistical test.

Group 3 was composed of 28 American varieties of watermelons (listed in table II), and group 4 contained 10 American and 79

TABLE II

MEAN SHAPE INDICES OF COTYLEDONS, SEEDS, AND FRUITS OF 28 AMERICAN COMMERCIAL VARIETIES OF WATERMELONS

VARIETY	COTYLEDONS		SEEDS		FRUITS	
	N	SHAPE INDEX	N	SHAPE INDEX	N	SHAPE INDEX
Angeleno, White Seeded	18	697± 5	20	602±6	6	877±16
Arikara	24	668± 6	20	593±5	7	827±17
Chilian, Black Seeded	25	685± 5	20	592±4	5	923±25
Chilian, White Seeded	26	693± 4	20	607±4	7	924±14
Climbing	18	700± 8	20	651±7	5	993± 5
Cole's Early	20	693± 6	20	621±7	7	829±29
Excel	28	580± 7	20	592±5	7	552±18
Field's Jumbo	28	680± 4	20	605±6	6	803±14
Florida Favorite	18	601± 6	20	573±5	5	502±12
Fordhook Early	17	734± 6	20	601±6	7	832± 5
Golden Honey	17	698± 7	20	614±6	7	812±27
Ice Cream	21	653± 5	20	592±5	5	504±19
Kleckley Sweet	29	628± 4	20	605±5	5	489±13
Klondike	24	685± 6	20	630±4	3	705±33
Mammoth Ironclad	21	683± 5	20	586±5	6	600±19
Mountain Sweet	23	672± 6	20	603±5	6	878±20
New Wonder	17	597± 5	20	591±6	4	552± 8
Phinney's Improved	14	696± 8	20	597±4	5	632± 9
Princess	22	651± 4	20	665±8	7	952±12
Rattlesnake	26	584± 6	20	561±6	5	611±29
Stone Mountain	20	690±11	20	607±6	6	903±37
Sugar Stick	23	556± 6	20	593±6	7	497±13
Sun, Moon, and Stars	23	662± 6	20	621±4	7	817±46
Sweetheart	22	684± 5	20	642±6	6	856±24
Sweet Siberian	19	623± 8	20	574±4	5	773±58
Thurmond Gray	20	636± 6	20	575±5	5	536±11
Will's Sugar	24	643± 5	20	647±8	6	904±18
Winter	19	707± 4	20	662±6	7	968±10

foreign varieties. Group 5 was the combination of groups 3 and 4. The correlation coefficients of these groups were lower than those for Iowa Belle (table I). It should be pointed out that the number of individuals listed under "fruits" in table II applies to both cotyledons and fruits for the correlation of shape of cotyledons with shape of fruits. Measurements from plants grown in the greenhouse were

added to those from the field to obtain the numbers under "cotyledons" in table II.

Group 6 was a miscellaneous lot of inbreds consisting of three selections of Kleckley Sweet, one of a Japanese variety, and two of a French variety. The 82 plants of group 7 represented the F_1 progenies of 14 different varietal crosses. Group 8 contained F_2 plants of hybrids between Iowa King and Iowa Belle and between Pride of Muscatine and Iowa Belle. The coefficients for total correlation and for correlation between selections of these hybrids are very high.

Group 9 was the combination of all the other groups of watermelons except group 2. In this total number of 1077 plants, coefficients for the correlation between indices of cotyledon shape and fruit shape were as follows: total correlation, 0.61; between varieties, 0.75; within varieties, 0.20.

The fact that the correlation between varieties in watermelons was generally high, whereas that within varieties was low, indicates that for each variety there is a characteristic cotyledon shape associated with a characteristic fruit shape, but that within these varieties there are many individual variations in shapes of cotyledons and fruits which are unrelated. The general impression which one gets in observing and working with the plants coincides with these results. The data indicate that it is possible to forecast the mean shape of fruits of a variety or selection by averaging a number of cotyledon indices, but that the probability of making accurate predictions for individual plants is considerably smaller. In practical experience, it has been possible to predict the general shape of fruits of selections of Iowa Belle merely by observing the shapes of the cotyledons without making measurements. Also, the homozygosity of fruit shape in a selection can be foretold by observing the uniformity of cotyledon shape.

CORRELATION IN CITRONS AND WATERMELON-CITRON HYBRIDS.—In all groups of watermelons, indices of the cotyledon shapes were significantly and positively correlated with the indices of fruit shapes. This was not true in the citrons studied. In 59 plants, representing 13 varieties, the correlation coefficients were practically zero (table I, 10). In a group of watermelon-citron hybrids the coefficients were not significant (table I, 11).

CORRELATION IN CUCUMIS MELO.—The coefficient for the total correlation between indices of cotyledon shape and fruit shape in 55 plants of *Cucumis melo* was 0.27, which was statistically significant. The correlation between varieties and that within varieties was not significant (table I, 12). It is interesting to note that the correlation between varieties was larger, and that within smaller, than the total, as was the case in all groups of watermelons. This group of cantaloupes was a very heterogeneous one and included plants from 19 different varieties.

CORRELATION OF SHAPE OF SEEDS WITH SHAPE OF COTYLEDONS AND WITH SHAPE OF FRUITS

The correlation between shape of cotyledons and shape of fruits in watermelons suggested that these characters might also be correlated with the shape of the seeds from which the plants were grown. Shape indices were calculated for 20 seeds from each of the 28 varieties listed in table II. Mean indices of cotyledons were obtained by averaging the indices of a number of plants grown in the greenhouse with those grown in the field. The correlation coefficient was calculated from these mean shape indices of cotyledons and seeds. This coefficient was 0.42 and was statistically significant. The mean shape indices of seeds (table II) were paired with the mean shape indices of fruits to determine the degree of correlation between shape of seeds and shape of fruits. The coefficient (0.60) was highly significant, indicating that varieties of melons with spherical fruits have seeds which are less elongated than those from long-fruited varieties.

Summary

1. The correlation of shape of cotyledons with shape of fruits, of shape of seeds with shape of fruits, and of shape of seeds with shape of cotyledons was investigated in melons.

2. Coefficients of correlation were calculated by using shape indices. By employing the methods of analysis of covariance, coefficients were determined, not only for the total correlation of variations in shape but also for the correlation between varieties and for that within varieties.

3. Shape of cotyledons was significantly and positively correlated

with shape of fruits in watermelons, the coefficients for the total correlation ranging from 0.40 to 0.75 and those for the correlation between varieties from 0.60 to 0.99.

4. There was no significant correlation of shape of cotyledons with shape of fruits in citrons nor in watermelon-citron hybrids.

5. Shapes of cotyledons were found to be significantly correlated with shapes of fruits in a small group of plants of *Cucumis melo* L. ($r=0.27$).

6. In 28 American varieties of watermelons, the mean shape indices of seeds were significantly correlated with mean shape indices of cotyledons ($r=0.42$) and with mean shape indices of fruits ($r=0.60$).

7. A significant change in the shape of watermelon cotyledons occurred as the seedlings grew, the relative length of the cotyledons increasing more rapidly than the width.

8. In many varieties of watermelons, and particularly in the Iowa Belle variety, fruit shape can be foretold from observations on the cotyledons. There is much greater accuracy in forecasting the mean shape of fruits of a variety or selection than in forecasting the fruit shape for an individual plant. Strains homozygous for fruit shape may be selected by observing the uniformity of cotyledon shape.

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SETCREASIA BREVIFOLIA, A FURTHER EXAMPLE OF POLYPLOIDY AND STRUCTURAL HYBRIDITY IN THE TRADESCANTIAE

M. MARGARET RICHARDSON

(WITH SEVEN FIGURES)

Introduction

The occurrence in the Tradescantiae of polyploidy and of structural hybridity, in some cases associated together, is well known (for example, *Rhoeo discolor*, *Zebrina pendula*, *Tradescantia bracteata*, and *T. virginiana*) and has been fully discussed by DARLINGTON (**2**), KOLLER (**8**), and others. The observations recorded here show that a form of *Setcreasia brevifolia* Rose exhibits both tetraploidy and structural hybridity, with configurations strikingly similar to those of *T. virginiana* (**2**, **8**).

The large size of the chromosomes, together with the small number and terminal position of the chiasmata in this material, makes this species suitable for a quantitative analysis of chromosome behavior in polyploid associations.

Material and methods

Chromosome observations in meiosis and in the first division of the pollen grain were made from aceto-carmine and smear preparations. The latter were fixed in 2 BE and stained in gentian violet. I am indebted to Mr. S. O. S. DARK for his illustrations of meiosis in the diploid (×4470) and of metaphase chromosomes in pollen grains (×3300). Illustrations of meiotic chromosomes in the tetraploid were drawn from aceto-carmine preparations at a magnification of ×3300.

The origin of the tetraploid form is unknown, but it is undoubtedly a member of the genus *Setcreasia;* and although morphologically distinct from the diploid form of *S. brevifolia*, being smaller in habit, it agrees with this species more closely than with any other member and may therefore be considered a variety. *Setcreasia brevifolia* has been variously named *Treleasia* and *Neotreleasia brevifolia*.

Observations

Somatic chromosomes

There are two forms of *Setcreasia brevifolia;* one form has $2n = 12$ and the other is a tetraploid with $2n = 24$. The chromosomes are large with median or submedian spindle attachments, and show little variation in size. Metaphase chromosomes in the first division of the pollen grain are shown in figures 1 and 2. DARLINGTON (2, p. 214) examined somatic metaphase divisions in the root tip of the tetraploid form, and reports that the chromosomes closely resemble some varieties of *Tradescantia virginiana,* but that there are three or four chromosomes with constrictions more nearly terminal than is usual in that species.

TABLE I

CHIASMA FREQUENCY AND TERMINALIZATION COEFFICIENT IN THE DIPLOID

	No. of nuclei	No. of bivalents	Total no. of chiasmata	Mean no. of chiasmata per nuclear complement	Mean no. of chiasmata per bivalent	Total no. of terminal chiasmata	Terminalization coefficient
Diakinesis.....	5	30	86	17.2	2.86	20	0.23
Metaphase.....	4	24	63	15.75	2.62	36	0.57

Meiosis in the diploid

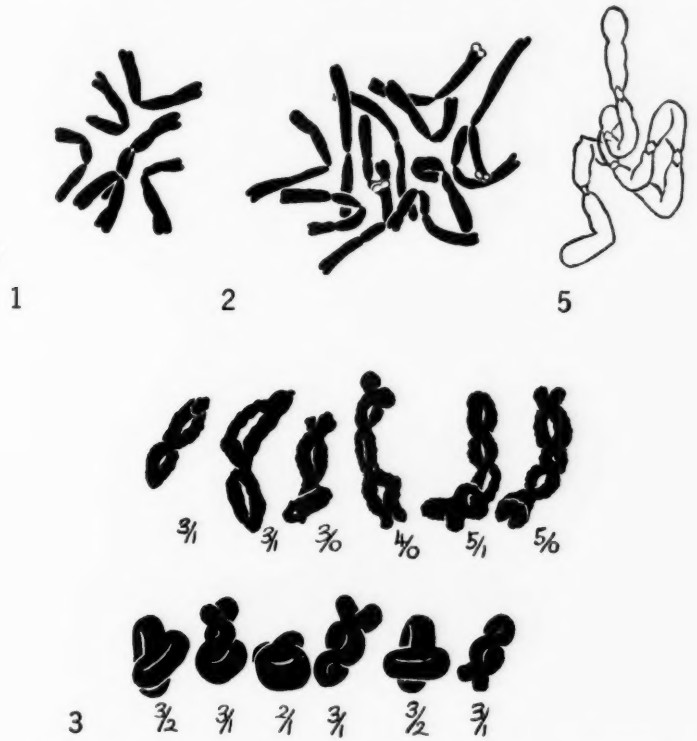
Meiosis was regular in the diploid form (fig. 3), and configurations very similar to those of *Spironema fragrans,* another member of the Tradescantiae, were found at diakinesis and metaphase (11).

The number of chiasmata varies from 3 to 5 per bivalent at diakinesis, with a mean of 2.86. There is a slight reduction in the number of chiasmata at metaphase, and the proportion of terminal associations is increased (table I). No attempt was made to distinguish between different kinds of bivalents.

Meiosis in the tetraploid

Meiotic behavior in the tetraploid form is strikingly similar to that in *Tradescantia virginiana,* both in size and form of metaphase configurations and in the average number of quadrivalents per

nuclear complement. It differs from *T. virginiana* in that there is a higher degree of interstitial chiasmata and that fewer types of quadrivalent and trivalent associations requiring quadruple and triple chiasmata are present. Interstitial chiasmata are sometimes found in *T. virginiana*, but only comparatively infrequently, and



FIGS. 1–3, 5.—Fig. 1, first pollen grain division (n=6); fig. 2, same (n=12); fig. 3, meiosis in diploid form (diakinesis above, metaphase below); fig. 5, chain of 8 in the tetraploid form.

have been regarded by DARLINGTON (2) and KOLLER (8) as evidence of non-homology of parts of pairing chromosomes. DARLINGTON reports that three or four quadrivalents with six or four bivalents were most commonly found to comprise the nuclear comple-

ment in *T. virginiana;* all variations from one to six quadrivalents were present, and a few univalents, usually associated with trivalents, were observed. In *Setcreasia brevifolia* all variations of association from five quadrivalents and two bivalents to eleven bivalents and two univalents were seen, there being most frequently two or three quadrivalents. Trivalents are present only in small numbers (table II) and the number of univalents greatly exceeds the number of trivalents. One or two examples of interlocking were found (fig. 4).

TABLE II

FREQUENCY OF DIFFERENT TYPES OF CHROMOSOME ASSOCIATION IN THE TETRAPLOID

Total no. of cells	No. of chromosomes	Types of association with different numbers of chiasmata									
		viii		vi	iv		iii		ii		i
		8	7	6	4	3	3	2	2	1	
72	1728	1	1	1	96	67	1	28	229	190	129
Total		2		1	163		29		419		129

The relative scarcity of multiple chiasma associations is correlated with a low chiasma frequency and may indicate interference (tables III, IV). Only one bivalent with three chiasmata was seen in about 100 nuclei. One example of a quadruple chiasma was found, and six of triple chiasmata; four in quadrivalents and two in trivalents. These all required the formation of three chiasmata in a chromosome (figure 6 illustrates the types of chromosome association observed). Thus only six chromosomes out of a total of 1728 in the 72 cells analyzed showed the presence of three chiasmata, the remainder having two, one, or none (table IV). Quadrivalents, with the preceding exceptions, were found to be either rings or chains (table II) of roughly equal proportions, and trivalents were chains. Ring quadrivalents most commonly segregate disjunctionally, but several cases of two adjacent chromosomes going to the same pole were observed. Figure 5 shows one case of a chain of eight chromosomes.

FIG. 4.—Meiosis in the tetraploid form; whole nuclei showing different types of chromosome association in side views of metaphase, and numbers of whole chiasmata.

In following chiasma behavior quantitatively, the method used by DARLINGTON and MATHER (5) for triploid *Tulipa*, whereby "half chiasmata" are counted, has been adopted. Chiasma frequency is thus analyzed for each chromosome and not for different configurations. There are two possible ways of scoring the frequency of half

TABLE III

HALF CHIASMA FREQUENCY FOR WHOLE NUCLEAR COMPLEMENT AND PROPORTION OF TERMINAL ASSOCIATIONS OF WHOLE CHIASMATA (TETRAPLOID)

TOTAL NO. OF NUCLEI	TOTAL NO. OF CHROMOSOMES	HALF CHIASMA FREQUENCY FOR WHOLE NUCLEAR COMPLEMENT												TOTAL NO. OF HALF CHIASMATA	MEAN NO. OF HALF CHIASMATA PER COMPLEMENT	MEAN NO. OF HALF CHIASMATA PER CHROMOSOME	TOTAL NO. WHOLE CHIASMATA	TOTAL NO. WHOLE TERMINAL CHIASMATA	TERMINALIZATION COEFFICIENT
		26	28	30	32	34	36	38	40	42	44	46							
72	1728	..	1	3	6	14	20	11	6	10	1	..		2628	36.3	1.51	1314	1265	0.96

TABLE IV

PERCENTAGE FREQUENCIES OF CHROMOSOMES WITH DIFFERENT NUMBERS OF HALF CHIASMATA (TETRAPLOID)

TOTAL NO. OF CHROMOSOMES	TOTAL NO. OF HALF CHIASMATA	HALF CHIASMA FREQUENCY			
		0	1	2	3
1728	2628	7.4	33.3	58.8	0.3

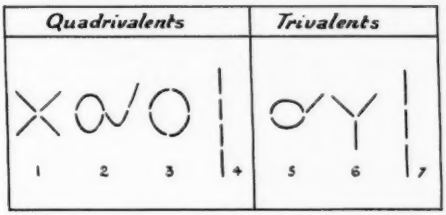
chiasmata, according to the genetical interpretation of a chiasma; these have been adequately discussed by DARLINGTON and MATHER. In the present analysis, half chiasmata have been counted in agreement with the modified chiasmatype theory (3). Thus a chain of four is scored as having 1, 2, 2, 1 half chiasmata per chromosome, and a quadrivalent of the type 2 (fig. 6) 1, 2, 3, 2 half chiasmata per chromosome (fig. 7).

Dissimilar chromosomes are not distinguishable during meiosis,

so that they have all been classed together; but since only small size differences are present, they may be expected to show similar chiasma frequencies. It is found that there is a mean half chiasma frequency of 1.5 per chromosome (table III). The number of chiasmata per chromosome is therefore much reduced from the diploid.

Evidence of structural hybridity

The probable presence of structural hybridity in this tetraploid form is shown, not only by associations of more than four inter-



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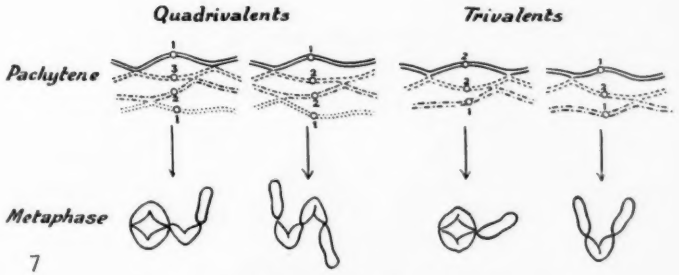

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Figs. 6, 7.—Diagrams illustrating half chiasmata

stitial chiasmata and low chiasma frequency, but also by the presence of trivalents and the large excess of univalents. Although chiasma frequency is unknown at the early stages, it may be deduced to be low from the infrequency of multiple chiasmata.

Trivalents are not usually found in pure autopolyploids, or only very rarely, and potential quadrivalents are replaced by bivalents and univalents (*Primula sinensis*, 3; *Solanum lycopersicum*, 9; *Solanum esculentum*×*racemigerum*, 1; *Avena*, 4). On the other hand

trivalents are present in small proportions in tetraploid interchange heterozygotes (*Tradescantia virginiana, Rosa relicta,* **6**; *Anthoxanthum odoratum,* **7**; *Aucuba japonica,* **10**).

If pairing is complete and there is only one exchange of partner between the four chromosomes in an autotetraploid, trivalents may be formed only in two-thirds of the cases when there are two chiasmata per association; or when there are more than two chiasmata, if two lengths of paired chromosomes fail to form chiasmata and one chromosome has all the chiasmata in the association. If one length of paired chromosomes is sufficiently long to form two or more chiasmata, it seems unlikely that the other arm of equal length in this configuration will fail to form any. Similarly, if there are two exchanges of partner between the four chromosomes, trivalents may be formed only in cases when the chiasma frequency per association is low and there are three or four paired lengths of chromosomes without chiasmata.

When four pairing chromosomes are structurally differentiated, the frequency of trivalent formation may be increased for two reasons: (1) Greater mechanical difficulties of pairing, leading to unsynapsed parts of chromosomes and hence reduction in the number of pairing blocks. (2) A greater variation in pairing, for if structural interchange between chromosomes took place previous to chromosome duplication, there will be four pairs of similar chromosomes of the following constitution: 2 AA′, 2 AB′, 2 BA′, and 2 BB′. Or if subsequently, there will be two sets of three similar chromosomes and two dissimilar chromosomes: 3 AA′, 1 AB′, 1 BA′, and 3 BB′. In both cases increased opportunities for trivalent formation are found. There is no evidence to show which has occurred.

The presence of single interstitial chiasmata instead of loops indicates that the interchanges are probably at the ends of the chromosomes. The occurrence of four interstitial chiasmata in a single nucleus, and the ring of eight, show further that there was at least one interchange, if this took place before the number of chromosomes was duplicated; or that there have been more, if structural hybridity has followed tetraploidy.

In view of the differences in cytological behavior, it seems probable that the tetraploid form is specifically distinct from the diploid.

Summary

1. Meiosis is followed in two forms of *Setcreasia brevifolia* Rose. Behavior is normal in the diploid ($2n = 12$) and bivalents show five to two chiasmata at diakinesis, with a slight reduction in number at metaphase and an increase in the number of terminal associations.

2. Behavior in the tetraploid is very similar to that in *Tradescantia virginiana*, and the same types of associations are found Chiasma frequency is reduced, and potential quadrivalents are replaced by a small proportion of trivalents, bivalents, and univalents.

3. Structural hybridity is indicated by associations of more than four, by interstitial chiasmata, and by trivalents.

4. A quantitative analysis is made of the behavior of the tetraploid from 72 nuclei.

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MORPHOLOGY OF AN INTERNAL TYPE OF ABNORMALITY IN THE FRUIT OF THE PEPPER[1]

H. L. COCHRAN

(WITH FOURTEEN FIGURES)

Introduction

The fact that an internal type of abnormality (fig. 1) occurs rather frequently in the fruit of the pepper, *Capsicum frutescens* L., was pointed out by the writer (2) in a previous publication. Since this

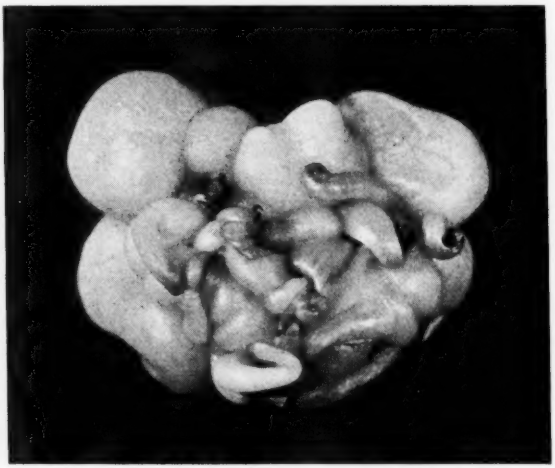


FIG. 1.—Fifteen separate and distinct internal abnormalities from a single pepper fruit.

time a morphological study of the abnormality has been undertaken, primarily to determine, if possible, something of its origin and ontogeny. Facts bearing on this problem seem of economic importance from the point of plant improvement, for such affected fruits usually develop parthenocarpically. Apparently no similar morphological

[1] Contribution no. 123 from Department of Vegetable Crops, Cornell University.

study of the pepper fruit has been reported, although descriptions of the gross morphology of this peculiar malformation do appear in the literature (**18**, **1**, **10**, **17**, **7**, **13**, **6**, **11**, **19**, **15**, **12**, **8**, **2**).

Material and methods

The material for this study was selected from pepper plants grown under controlled temperature conditions (60°–70° and 70°–80° F.) in the greenhouses of the Department of Vegetable Crops during the winter of 1932–33. Collections were made at intervals ranging from the early bud stage until fruit maturity, in order that any existing internal abnormalities might be found in their various stages of development. Karpechenko's chrom-acetic killing and fixing solution was used with good success in all cases. The usual laboratory method of dehydrating and imbedding the material in paraffin was followed. Slides were made of sections cut 8–12 μ thick and stained with Heidenhain's iron-alum haematoxylin. All drawings were made with the aid of a Spencer type camera lucida.

Investigation

Gross morphology of normal pepper fruit

The young bud emerges as a mere protuberance from meristematic tissue in the axils of branches of the plant. As soon as the bud begins to open an aperture is formed at its tip, where the corolla lobes meet. The aperture gradually increases in size and extends downward along the margins of the lobes. Soon after this the lobes spread out, thereby exposing for the first time the immature ovary. This becomes larger and is characteristically shaped according to the variety. The fruit matures in approximately 60 days from anthesis, at which time it possesses a distinct depression at the base and three external furrows, one between each locule, that extend down the sides of the fruit to the apical end. From 300 to 550 seeds, depending on the size of the fruit, are borne on the placental tissue, the number usually being rather equally distributed in each of the three locules.

Origin of abnormality

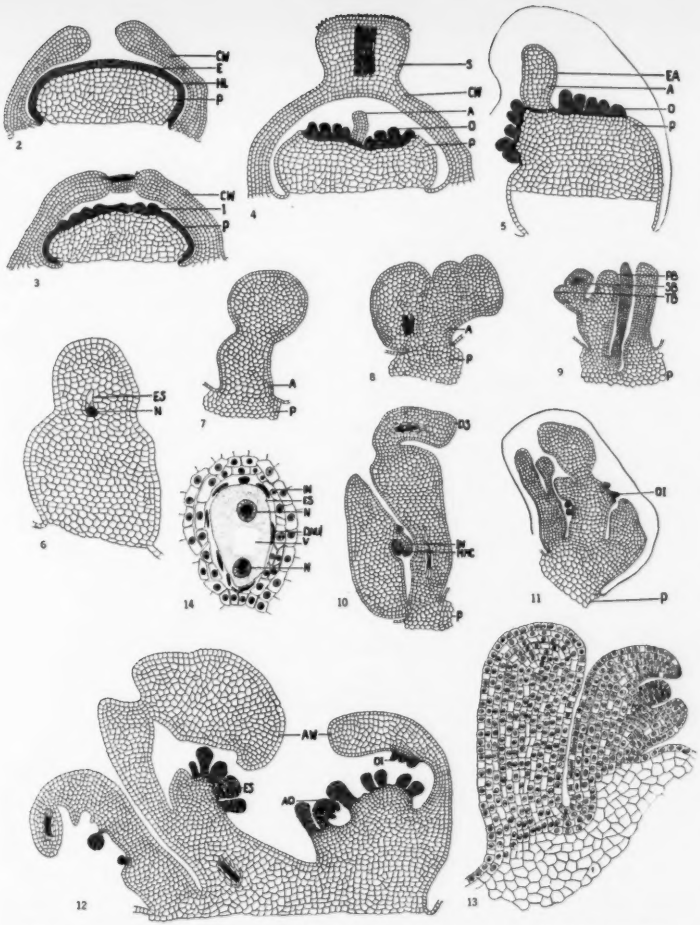
The internal abnormality is hypodermal in origin, being initiated in the outermost layers of cells of the placenta when the pepper bud

ranges from 1 to 3 mm. in diameter. It develops simultaneously with the ovules and for a while approximates them in most respects. Immediately preceding the development of the abnormality and ovules, the epidermis of the placenta preserves a uniform surface (fig. 2). The cells of the epidermis and a layer of hypodermal cells soon differentiate from the rest of the placental tissue. The cytoplasm of these cells is very dense, and each contains a conspicuous nucleus which nearly fills the entire cell. These cells stain more deeply than do the adjoining ones of the placenta. It is evident that the abnormality initial differentiates from the subepidermal tissue, and at this stage cannot be detected from the ovule initials that differentiate in groups from the same tissue. The abnormal tissue, as well as the ovules, divides at first only in one direction. The epidermal cells divide anticlinally. The cells lying between the abnormal initials and those of the ovules divide less rapidly and soon become compressed and distorted. At this stage the placenta loses its evenness and becomes wavy in outline. The identity of the many ovules and that of the abnormality as well is manifested as blunt protuberances on the placenta (fig. 3). Subsequent development of the abnormality and ovule initials is very dissimilar. The ovulary tissue now divides in all directions and soon constitutes the beginning of the nascent ovules, while the malformed tissue develops into a protuberance which constitutes the ensuing abnormality. The accompanying ovules abort almost invariably.

ONTOGENY

The young developing abnormality is distinguished early in its life history by the fact that it grows at an unusually rapid rate as a mass of lightly stained undifferentiated cells. The epidermis, however, which consists of a single layer of small, rather isodiametric cells, is differentiated soon thereafter (figs. 4, 5). The remainder of the abnormality is made up of a mass of thin walled parenchyma tissue (2). As the abnormality increases in size and cell numbers, its rate of development decreases; yet it remains in excess of any accompanying ovules.

Cell division does not continue at a very constant rate throughout the abnormal tissue. Because of this various forms are developed



FIGS. 2–14.—Fig. 2, longisection through pepper bud 1.5 mm. in diameter showing uniform placental surface prior to ovule and abnormality initiation. ×178. *cw*, carpel wall; *e*, epidermis; *hl*, hypodermal layer; *p*, placenta. Fig. 3, longisection of pepper bud 3 mm. in diameter showing ovule and abnormality initials. ×178. *i*, initials of ovules and abnormality. Fig. 4, longisection through young ovary showing immature ovules and developing abnormality. ×178. *s*, style; *a*, abnormality; *o*, ovules. Fig. 5, longisection of young ovary showing developing abnormality with epidermis differentiated. ×178. *ea*, epidermis of abnormality. Fig. 6, longisection of developing abnormality resembling somewhat a normal ovule in shape and containing an abnormal uninucleated embryo sac. ×750. *es*, embryo sac; *n*, nucleus. Figs. 7, 8, longisections through two abnormalities showing close resemblances of abnormal and placental tissue. ×100. Fig. 9, longisection of abnormality showing primary, secondary, and tertiary branches. ×100. *pb*, primary branch; *sb*, secondary branch; *tb*, tertiary branch. Fig. 10, longisection through developing abnormality showing distorted style and abnormal ovule in megaspore mother cell stage. ×178. *ds*, distorted style; *in*, integument; *mmc*, megaspore mother cell. Fig. 11, longisection of abnormality showing ovule initiation therefrom. ×178. *oi*, ovule initials. Fig. 12, longisection through rather mature abnormality showing ovule initiation from several points, an anatropous ovule, and a 2-celled embryo sac. ×415. *aw*, abnormality wall; *oi*, ovule initials; *ao*, anatropous ovule; *es*, 2-nucleate embryo sac. Fig. 13, enlarged view of anatropous ovule (taken from fig. 12). ×960. Fig. 14, enlarged view (from fig. 12) of 2-nucleate embryo sac from within the abnormality, showing integument, disintegration of nucellus, nuclei, and vacuole. ×960. *dnu*, disintegrating nucellus; *v*, vacuole.

and no definite shape can be assigned them. As far as could be detected, the malformation is always initiated as a single protuberance which later may or may not branch (figs. 7–12). It is not uncommon to find abnormal tissue that branches into two or three portions, some of which subsequently develop similar secondary and tertiary branches. Various other grotesque shapes may appear. One abnormality was found which somewhat resembled a normal ovule containing an abnormal one-celled embryo sac (fig. 6). Some abnormalities produce no style-like structures, while others produce two or even three of them.

A microscopic study of prepared sections and also of living specimens with the wide-field binocular microscope indicates that the abnormality is anatomically the same in most respects as the placenta from which it is derived. Both consist of similar parenchyma tissue that has the same stain reaction and both are capable of initiating and developing ovules. The ovules of the abnormality, however, are not restricted to any particular portion of the tissue but appear to arise at any point thereon (figs. 11, 12). Some of these ovules are apparently normal, as far as size, shape, structure, and certain stages of development are concerned. While it is true that many of the ovules from within the abnormality abort when young, those that develop further are anatropous (figs. 12, 13). This work has revealed but one ovule in what appears to be the megaspore mother cell stage (fig. 10), and none developed further than the 2-nucleate embryo sac stage (fig. 14). Continued development probably occurs rather rarely; at least the ovule rarely reaches maturity. Apparently STURTEVANT (**17**) is the only person who has reported such an internal type of abnormality which contained seed.

Normal and abnormal 1- and 2-nucleate embryo sacs have been compared and found to be very similar. Even the nucellus of both start degenerating upon reaching approximately the same stage of development. The only exception noted was a difference in time of nuclear division. When using ovule size as a criterion of age, the one from within the abnormality was found to be rather slower in dividing, although the two subsequently became the same.

Discussion

For the past three years the type of internal abnormality reported here has been prevalent to some extent in fruits from plants grown in the greenhouse under controlled temperature conditions. A study has been made of these changes but still the question arises, What explanation can be offered as to the cause of this phenomenon? No observations made in the present study supply a definite answer to this question.

As the affected pepper fruits for the most part develop parthenocarpically, and are therefore partially sterile, the same factor or interrelation of factors may be responsible for both the seedless condition and the development of the abnormality as well.

DARWIN (3) has pointed out that influences of both environment and hybridity may, through compensations of growth, result in such a condition as seedless fruits. The characters parthenocarpy and embryo abortion in seedless fruits are known to be hereditary, as exemplified in the cucumber by WELLINGTON and HAWTHORN (20) and HAWTHORN and WELLINGTON (9). According to PEYRITSCH (14), however, these conditions may be induced artificially.

Since the plants used in this study were grown in the greenhouse under almost ideal conditions as far as temperature, soil nutrients, and soil moisture are concerned, some vegetative vigor resulted. There seems to be no direct evidence that the seedless type of fruits containing the abnormality arises as a direct response to excessive vegetative vigor, nevertheless ERNST (5) has considered that such characters as apogamy and seedlessness may be initiated under such conditions. STOUT (16) is of the opinion that frequently certain of the unfavorable environmental factors to which a plant is subjected may become sufficiently regular in action to institute a type of sterility. Thus in respect to nutrition there may be a response to such influences as an excess of nitrogen or of water. In general this is characterized by excessive vegetative vigor.

While the preceding evidence on sterility affords no direct explanation for the occurrence of the internal type of abnormality, it is believed that the evidence is of enough significance to suggest that hybridity and vegetative vigor as affected by environmental factors may be involved.

Summary

1. The internal type of abnormality occurs frequently in the fruit of the pepper.

2. The abnormality is hypodermal in origin and develops for a while simultaneously with the accompanying ovules. It is initiated when the pepper bud ranges from 1 to 3 mm. in diameter.

3. The young abnormality is distinguished early in its ontogeny by a group of undifferentiated, lightly staining cells which divide very rapidly. The rate of growth, however, decreases with increase in size and age.

4. The epidermis, which consists of a single layer of rather isodiametric cells, differentiates soon after the preceding stage.

5. No definite shape can be assigned the abnormality since it is so variable. Some contain styles while others do not.

6. The abnormality and the placenta from which it is derived are anatomically the same; both consist of similar parenchyma tissue that takes the same stain, and both are capable of initiating and developing ovules.

7. Most ovules borne in the abnormality abort but those that survive are typically anatropous.

8. One ovule from within the abnormality was found in the megaspore mother cell stage and none were found any further advanced than the 2-celled embryo sac stage.

9. Nuclear division of the embryo sac takes place somewhat slower than that of a normal embryo but subsequently they become identical.

Grateful acknowledgment is made to Dr. ORA SMITH for valuable suggestions and criticisms during the course of this study.

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MICROINCINERATION STUDIES: II. LOCALIZATION OF ASH-YIELDING SUBSTANCES DURING MEIOSIS AND ITS POSSIBLE SIGNIFICANCE IN X-IRRADIATION PHENOMENA[1]

FRED M. UBER AND T. H. GOODSPEED

(WITH FOUR FIGURES)

Using the improved microincineration technique of POLICARD (**6**), SCOTT (**7**) demonstrated the disposition of inorganic residues in the male germ cells of the white rat and found the major portion of the ash localized in the chromosomes. Later KRUSZYNSKI (**5**) and others made further observations on several types of cells and on smaller cytological entities with varying success. Previous to these animal studies, FUNAOKA and OGATA (**1**) had obtained similar results with pollen mother cells of *Vicia faba* in the early stages of division, but their bright-field photomicrographs, taken at a low magnification, combined with their very brief descriptions, give an incomplete and unsatisfying picture. From several standpoints a more detailed investigation of the topography of the dividing plant cell in terms of the disposition, amount, and character of the inorganic salts appeared highly desirable. The bearing of such evidence on the interpretation of the lethal effects of x-rays, its significance for various cytogenetic phenomena, and its intimate relation with the physicochemical organization of the cell immediately come to mind.

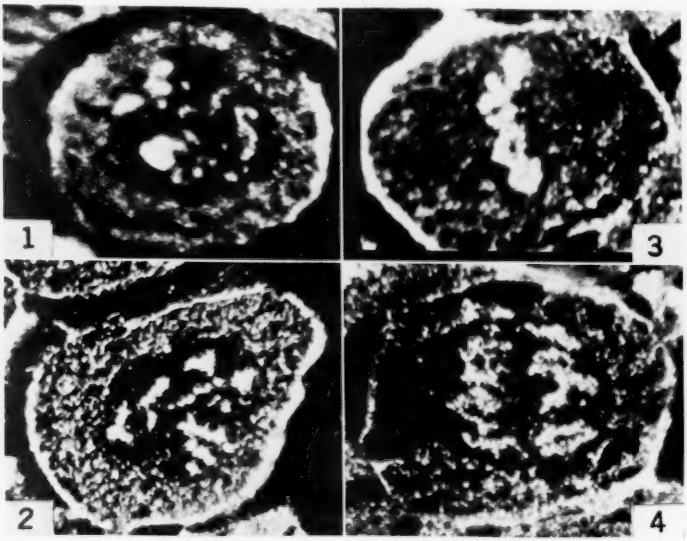
Anthers of *Lilium longiflorum* var. *grandiflorum* and of *Kniphofia* sp. were fixed by the Altmann freezing-drying technique, imbedded in paraffin and cut at 7 μ. The merits of this method of fixation as a pretreatment for microincineration as well as a full discussion of the technique itself were presented in previous publications (**2**, **3**, **4**), to which the reader is referred also for typical photomicrographs of control material. Sections to be incinerated were placed on microscope

[1] Investigation supported by a grant from The Rockefeller Foundation. The writers are also indebted to Miss ALICE HANDSCHIEGL, who conducted a number of preliminary investigations.

slides without the aid of an adhesive, whereas controls were stained with Heidenhain's iron-alum haematoxylin in the traditional manner. The microincineration apparatus and technique were described at length in the initial paper of this series (**11**). Usually the time allowed to heat the sections from room temperature to 550° or 600° C. was two hours, the final temperature being maintained for a further period of 2–3 hours. The slides were removed from the oven while hot and the edges sealed at once in order to exclude moisture from the hygroscopic ash. The bicentric dark-field condenser of Leitz with direct current arc illumination was utilized for both visual study and photomicrography, the latter by means of the Leitz "Makam" camera.

The topographical disposition of the inorganic residue following incineration in PMC of *Lilium* is shown in figures 1–4. Striking characteristics of the ash picture are (a) absence of ash in the karyolymph, (b) abundance of mineral residue in the chromosomes, (c) presence of ash in the cytoplasm, and (d) presence of ash in the cell wall. The ostensibly heavy ash deposits at the periphery of the cells are not wholly the product of the cell walls but are due in large measure either to the slime of the anther sac or to a partial collapse of the cytoplasm incident to its dehydration during the freezing process of fixation. Simultaneously certain intercellular spaces were thus created, as evidenced in figures 1–4 (*cf.* also **3**, fig. 1). In figures 1 and 2 the dense white ash of the late prophase chromosomes contrasts clearly with the black background representing the karyolymph. Although little is known concerning the chemical composition of the karyolymph, microincineration evidence suggests that it is radically different from that of the cytoplasm. Figures 3 and 4 represent the inorganic residues of the heterotypic metaphase and anaphase respectively. Save for the large quantity of ash in the chromosomes, the spindle regions are devoid of non-volatile mineral substances. A logical interpretation of this evidence would be that the spindle area is formed from the karyolymph of the preceding prophase, a conclusion many cytologists (**8**) have reached from independent considerations. Residues of telophasic nuclei showed no structure, in general, but only a continuous heavy deposit. The same was true of resting nuclei in the somatic cells of the anthers.

Incinerated sections of anthers of *Kniphofia* sp. gave results comparable with those obtained with *Lilium*, and in addition illustrated most graphically the rich ash content of the tapetum. The tapetum is generally recognized as a nutritive layer (**9**, pp. 250, 251), so that a high mineral content might well be anticipated. Whether the chromosomal and other ash consists mostly of salts of Ca, Mg, K,



FIGS. 1–4.—Dark-field photomicrographs showing ash distribution in pollen mother cells of *Lilium longiflorum*. Leitz bicentric condenser, N.A. 1.2. 810×. Sections, 7 μ, fixed by Altmann freezing-drying technique: Figs. 1, 2, diakinesis; nuclear cavity clearly delineated, revealing absence of ash in karyolymph; ash conspicuous in bivalents and present in cytoplasm. Fig. 3, heterotypic metaphase plate with ash in chromatin predominant over cytoplasmic residues. Fig. 4, heterotypic anaphase; details of chromosomes well preserved. Note outline of spindle region.

etc., as suggested by several investigators, or is due in large measure to phosphorus, has not as yet been determined. Thus far only gross chemical tests have been made, but these attest the abundant presence of the latter and indicate that a large part of the residue in both cytoplasm and chromatin may result from organic phosphorus compounds. No evidence is furnished on this point by

KRUSZYNSKI (**5**), who, in making a qualitative chemical analysis of incinerated nerve cells by means of the micromanipulator, apparently did not test for elements other than K, Ca, Fe, and Mg. It is to be noted that nucleic acid, which is known to be present in chromatin and is probably present also in the cytoplasm as a nucleoprotein (cf. **9**, pp. 58, 59), has a phosphorus composition of nearly 10 per cent.

The bearing of the fact that elements of comparatively high atomic number are concentrated in the chromosomes on the interpretation of quantitative lethal effects of x-radiation must now be considered. The customary measurement of x-ray dosage in roentgens is based upon the assumption that protoplasm simulates air as regards its mass absorption coefficients. But from the known composition of nucleic acid with its high phosphorus content and the demonstrated presence of non-volatile ash in the chromosomes, a mass absorption coefficient for chromatin at least double that of air would seem a conservative estimate. Although such increased absorption would probably be insufficient to account completely for the known greater sensitivity of nuclei as compared with cytoplasm, it might well be an important contributory factor. The microincineration evidence in itself does not constitute proof on this point, since nuclear injury may conceivably result in a number of ways. According to the quantum theory of biological effects from high frequency radiation, injury occurs when a specific number of quanta are absorbed in a "sensitive volume" of an irradiated object (for relevant literature, see UBER and GODDARD **10**). Should chromatin constitute such a "sensitive volume," then it follows that its size would be but a fraction of that calculated for it on the ordinary assumptions.

If the chromosomes possess a relatively great absorptive capacity for high frequency radiation owing to presence of elements of comparatively high atomic weight, then there should be an abrupt quantitative variation in the biological response to x-ray wave lengths on either side of the absorption discontinuities of such elements. Although both quantitative and qualitative wave-length effects have been sought many times in recent years, no investigator, to the writers' knowledge, has explored the "K" discontinuity region

of the elements calcium, potassium, and phosphorus. In the wavelength range concerned, 3–6 Angström units, the obtaining of critical data on biological material with monochromatic x-rays constitutes a difficult task, but one which the writers have planned to undertake in the immediate future.

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CURRENT LITERATURE

The Algae and Their Life Relations. Fundamentals of Phycology. By JOSEPHINE E. TILDEN. Minneapolis: University of Minnesota Press, 1935. Pp. xii+550. Illus. $5.

Instructors presenting a course on the algae have never had a comprehensive text-book in English to which they could refer the student for material to supplement the lectures and the laboratory work. Professor TILDEN'S book is designed to meet this need.

The first chapter is a presentation of the author's hypothesis that the five classes of algae (Cyanophyceae, Rhodophyceae, Phaeophyceae, Chrysophyceae, and Chlorophyceae) arose in successive eras, and that the characteristic pigmentation of each class was determined by the intensity of the earth's illumination during the era in which it appeared. The second chapter (Distribution of algae in time and space) divides time into the Cyanophycean, Rhodophycean, and other periods, and holds that variation in illumination is the factor of primary importance in the present-day distribution of marine algae in the Pacific Ocean. The third chapter stresses the characteristic pigmentation and food reserves among the various classes of algae. This chapter is the only place where distinctions are made between the classes of algae. It could have been improved by pointing out that a lack of sexual reproduction, or fertilization by non-motile male gametes, or a distinctive type of flagellation in planogonidia and gametes are of equal importance in distinguishing particular classes.

In the detailed consideration of the various classes, 51 pages are devoted to the Cyanophyceae, 105 to the Rhodophyceae, 129 to the Phaeophyceae, 15 to the Chrysophyceae, and 119 to the Chlorophyceae. The chapter on the Rhodophyceae exemplifies the strong and the weak features of the book. The descriptions of the morphology of the various genera take into consideration the most recent literature and present this accurately. Descriptions of the methods of harvesting and manufacture of such commercial products as "Irish moss" and agar add immeasurably to the chapter. They make the student realize that there is something more to the red algae than carpogonidia and trichogynes, or haplobionts and diplobionts. On the other hand, starting a discussion of reproduction in the Rhodophyceae with the Ceramiales makes a difficult subject even more difficult. The author's reason for this lies in her belief that the simplest plants in any group are the most recently evolved ones. It is difficult to find a justification for this novel hypothesis, either from the known geological history of other plants, or from that of animals. In connection with the account of each order there are technical descriptions of the various families and of several

genera in each family. There is no evident reason for the inclusion of these technical descriptions. The book is not designed to serve, and cannot serve, as a manual for the identification of algae. If the generic descriptions are intended to show the student the wide range in structure among the various orders, the same end could have been accomplished in a more effective manner by means of a general discussion. The details of reproduction are described for one or more genera in each family. Sometimes these are given *in extenso* and with numerous illustrations. At other times they are brief and illustrated by means of a life cycle diagram.

The chapters on the brown and the green algae are organized in the same manner and proceed from the complex to the simpler forms. The diatoms are given the same amount of space as the Chinese ideograph meaning algae (a full page text figure and a half page of text).

The author meets the student's confusion arising from the conflicting terminology in phycological literature by proposing a new terminology that is consistent throughout. The most striking innovations (pp. 236–237) are the restriction of the term spore to those produced as a result of meiosis, and the designation of all asexual reproductive bodies as gonidia. Examples of the latter are planogonidia (zoospores), aplanogonidia (aplanospores), and carpogonidia (carpospores). Admitting its greater consistency, such a wholesale introduction of new terms can only further obscure the condition that it aims to clarify. It is to be hoped that the student will consult the papers listed in the extensive bibliography. If he does, he is certain to meet a different terminology and one that must be translated into the terminology of Professor TILDEN's book. Sometimes this will be a difficult matter. For example, one reading the literature on the Phaeophyceae is almost certain to run across the terms "unilocular sporangium" and "plurilocular sporangium." These are not mentioned in the index of the book under review and, as far as the reviewer can find, only once in the text (p. 237). A simpler solution of the whole difficulty would have been the inclusion of a glossary of conflicting terms.

Taken as a whole, the book is an accurate presentation of the present-day factual knowledge of the algae arranged in an unorthodox manner. One cannot deny an author the right to express an opinion contrary to that of a majority of phycologists. However, in a book designed for students it would have been advisable to indicate interpretations that are not in accordance with the opinion of other phycologists. A minor example of this is the treatment of *Botrydium*. The author includes this alga in the Chlorophyceae without hinting that all other phycologists place it in the Heterokonteae.—GILBERT M. SMITH.

Translocation of Solutes in Plants. By OTIS F. CURTIS. New York: McGraw-Hill Co., 1935. Pp. xiii+273.

This new book is probably one of the most important recent contributions to plant physiology. No one is better equipped to treat of the subject than is CUR-

TIS, who some 18 years ago began his researches on solute translocation and who has since encouraged so many other investigators to focus attention on this problem. The book is divided into seven chapters, each dealing with a specific part of the problem: the importance of translocation, the upward transport of organic matter in the phloem, the upward transport of nitrogen and salts through the phloem, the downward transport through the xylem, the criteria and methods of study, the mechanism of movement, and the possible relations between solute movement and plant growth.

The book is written in clear and stimulating style. CURTIS presents all arguments in each chapter, and even though one familiar with the work on translocation will disagree with many of the viewpoints presented, he will subscribe to the author's fairness in his treatment. Perhaps the weakest chapter is that dealing with the upward movement of salts and nitrogen in the phloem. Here, although he presents evidence opposing his views, CURTIS is definitely biased in favor of phloem movement, even though in a later chapter he concedes the possibility of xylem movement. Each chapter is filled with descriptions of experiments dealing with living plants, a pleasant change from the tendency to use mechanical set-ups.

By far the most important and certainly the most brilliant chapter is that dealing with the method of movement. In this chapter of some hundred pages, CURTIS reviews the various hypotheses which have been suggested. His criticisms of these are specific. The hypotheses of MÜNCH, CRAFTS, etc., are first interpreted in the favorable light of their respective authors, then criticised in a most complete and scientific manner. It was rather difficult for the reviewer, however, to leave this very critical method to read about the "protoplasmic streaming hypothesis" which CURTIS seems to favor. While he admits the many attendant difficulties, even pointing out on page 185 that protoplasmic streaming has never been demonstrated in functioning sieve tubes, still in his summarizing statement on page 234 he writes, "The protoplasmic streaming hypothesis seems at present best adapted to meet the requirements of transport and to demand the structure and conditions that obtain in phloem tissue." One is forced to wonder just how scientific is intuition!

The book includes thirteen figures and twenty-four tables, as well as twelve pages of selected literature citations. Each chapter is tersely summarized.—HARRY F. CLEMENTS.

Plant Physiology. By MEIRION THOMAS. Philadelphia: P. Blakiston's Sons and Co., 1935. Pp. vii+494. figs. 57.

This volume is a worthy addition to the list of summaries of this field which have appeared recently. The book, intended for students who already have a general knowledge of the subject, is arranged in a logical order. Part I is concerned with protoplasm, and also contains a discussion of enzymes. Absorption, translocation, and elimination of water, solutes, and gases are treated in part II.

There are chapters on the vacuolated cell as an osmotic system, soils, absorption of water and solutes by roots, transpiration, conduction of water, conduction of solutes, and gaseous exchanges between plants and the outside air. Part III considers nutrition and metabolism. There are chapters on photosynthesis, respiration, and metabolism, in which are discussed the general metabolic processes occurring in plant tissues, including the metabolism of the fats and proteins. It would seem better to emphasize the importance of fats and proteins by discussing the synthesis and the other metabolic features of these compounds in separate chapters, or at least in a prominent subsection of a chapter. Growth and plant movements are treated in part IV. The book contains three appendixes, two of which present the more advanced material on organic compounds and physical chemistry, amplifying considerably various portions of the text proper. Appendix III is comprised of 163 citations, which are referred to by number throughout the text. In connection with the discussions of most of the plant processes, there are descriptions of experiments that may be tried in measuring or demonstrating the process. Usually there is a figure of the apparatus to be used.

In a few places in the book the author fails to emphasize recent viewpoints. For example, the absorption of salts by the plant is described as a physical process in which the particles diffuse from a region of greater concentration in the soil solution to one of less in the plant. While this may be true in some cases, there is certainly enough recent work, especially in America, to show that in many cases the salts enter against the gradient and that the greater concentration on the inside is an effective one and is not due to the salts being adsorbed or tied up in some way with the cell contents. The reviewer noticed one or two obvious errors. For example, it is stated that cane sugar is laevo-rotatory, and that the mixture of the products of hydrolysis is dextro-rotatory, since glucose is dextro-rotatory to a greater extent than fructose is laevo-rotatory.

On the whole the book is a well balanced, thorough treatment of the principal physiological processes occurring in the green plant. It should be of great interest and help, not only to the general botanist and plant physiologist, but to workers in the various applied fields such as horticulture and agronomy.—S. V. EATON.

An Illustrated Manual of Pacific Coast Trees. By HOWARD E. MCMINN and EVELYN MAINO. Berkeley: University of California Press, 1935. Pp. xi+409. figs. 415.

This book should prove entirely usable both for botanists and for those generally interested in trees. Not only are the native trees characterized, but also more than 400 introduced species.

There are few places where a greater variety of environmental conditions are likely to be encountered than in the area covered by this book—California, Oregon, Washington, and parts of British Columbia, and it is therefore to be ex-

pected that a considerable number of introduced species of trees should be found there. It is helpful to have at hand a manual which makes the identification of these reasonably certain. A number of the more common fruit trees of greatest commercial importance are mentioned, but they are not characterized in detail sufficient for identification. This omission seems unfortunate, since such trees are as botanically unknown to many as are the various more or less ornamental forms which are included. Suggestions for the possible use of both the native and introduced forms as landscape material and the conditions under which the various species may be expected to thrive add greatly to the value of the book to the amateur gardener and professional landscape architect.

The keys are as simple as can be expected, and yet serve the purpose of distinguishing the species. The drawings are clear cut, and without cumbersome detail.—E. J. KRAUS.

British Stem and Leaf Fungi (Coelomycetes). Vol. I. By W. B. GROVE. New York: Macmillan & Co., 1935. Pp. xx+488. $7.

Two groups of the fungi which infect the stems and leaves of plants stand out prominently because of their economic importance. The first of these are the rust fungi (Uredinales) and the second the Coelomycetes, a group belonging to the "Imperfect Fungi," and including the Sphaeropsidales and the Melanconiales. The first group was dealt with in British Rust Fungi (Cambridge University Press, 1913). The second group GROVE now plans to present in two volumes. The first volume has recently appeared and includes those Sphaeropsidales which have colorless spores. The second volume will contain the Sphaeropsidales with colored spores and the Melanconiales.

Volume I includes the Hyalosporae, Hyalodidymae, Hyalophragmiae, and the Scolecosporae. Most of the species here treated have been seen and examined microscopically, and the account given of each species is in the main purely morphological. A complete host index and a binomial index are given.

The need for a work dealing with this group has been apparent for a long time. In the opinion of the reviewer this volume and the forthcoming one will effectively meet the need. With the keys, indexes, and species descriptions, the identification of these fungi should be made much more certain. Interest in the group should be materially stimulated, not only in Great Britain but among the students of these fungi throughout the world.—J. M. BEAL.

On the Flora, Including Vascular Land Plants, Associated with Monograptus, in Rocks of Silurian Age, from Victoria, Australia. By W. H. LANG and ISABEL C. COOKSON. Phil. Trans. Roy. Soc. Series B. No. 517. Vol. 224. Pp. 421–449. pls. 29–32. 1935.

Until recently the oldest well known floras were those of the Middle and Lower Devonian of Aberdeenshire, Scotland, and Elberfeld, Germany. Our information on fossil land plants has been extended into the Silurian by this recent

publication, which will on this account become a milestone in the history of paleobotany. It has been assumed that extensive land floras existed in the Silurian and probably earlier, but no reliable evidence had been found of Silurian plants until the localities in Australia were discovered.

The plants are unquestionably Silurian, occurring in immediate association with Silurian graptolites. These plants are the oldest definitely known vascular land plants. The authors describe two new genera, *Baragwanathia* and *Yarravia*, besides listing fragmentary branch systems, similar to the well known Devonian genus *Hostimella*. All remains of plants belonging to *Baragwanathia* can be included in one species, *B. longifolia*. They are represented by leafy shoots, in some cases bearing sporangia; by stems with incomplete leaf bases, leaf scars, or without remains of leaves; by stems with laterally placed branches that were possibly rhizomatous; and by specimens showing the structure of the vascular system. The plant was doubtless homosporous, and the position of the sporangia and their relation to the leaf bases suggest comparison with Lycopodiales rather than with Psilophytales. On the other hand, the stellate arrangements of the stelar xylem now known for *Baragwanathia* can be closely compared with that found in *Asteroxylon*.

The new genus *Yarravia* shows unbranched axes or stalks, with a large synangial fructification which appears to have included a small number of larger linear-oval sporangia. *Yarravia* seems to have had a peculiar type of fructification, suggestive of derivation from terminal groups of large sporangia of the kind known in Psallophytales. The genus contains two species, *Y. oblonga* and *Y. subsphaerica*.—A. C. NOÉ.

Rock Garden Plants. By CLARENCE ELLIOTT. New York: Longmans, Green & Co., 1935. Pp. 328. figs. 17. $3.

To those interested in rock gardens and to students of botany this very readable book should prove interesting. In a delightful style, a considerable number of species and hybrids (chiefly alpine) are described. The author has limited his discussion to plants which he has actually known, and mostly grown. Cultural directions, preparation of sites and soils, pleasing and displeasing characteristics, are generally given in detail. Genera are listed alphabetically and under these the several species and numerous hybrids. The nomenclature follows the 1934 edition of the Kew Hand List of Rock Garden Plants.—K. C. HAMNER.

The Design of Experiments. By R. A. FISHER. London: Oliver & Boyd, 1935. Pp. xi+252.

A book on a rather new subject has recently been written by FISHER. It is concerned with the planning of experiments. Certain sections of the author's well known book, Statistical methods for research workers, are concerned with the technique of agricultural experimentation, and the present book is really an expansion of these sections. After the introductory chapter, there follow three

chapters in which the principles of designing an experiment are illustrated by a psycho-physical experiment, one of Darwin's growth rate experiments, and an agricultural experiment. Questions of design have been the most thoroughly studied in the field of agricultural experimentation, and in later chapters are given descriptions of the principal designs which have been found successful in this field. The author also shows how these designs can be applied to other fields. The main designs described are: the Latin square, the factorial design in experimentation, and compounding. Other subjects discussed are the increase in precision by concomitant measurements and the null hypotheses. The book should be of help to the worker in any field of science. If one is to read the book with the greatest profit, however, he should be fairly well acquainted with the principles of statistics, as outlined in the author's preceding work.—S. V. EATON.

The Fossil Flora of Scoresby Sound, East Greenland. Part IV. By T. M. HARRIS. Kopenhagen: C. A. Reitzel's Forlog, 1935. Pp. 176. pls. 20.

Part IV of a book on the fossil floras of Scoresby Sound has just appeared. The four volumes of the publication deal with: Cryptogams, exclusive of Lycopodiales (part I); the seed plants incertae sedis (part II); the Caytonailes and Bennettitales (part III); and Ginkgoales, Coniferales, Lycopodiales, and isolated fructifications (part IV). The fifth volume is intended to contain the stratigraphic relations and ages of the floras. The entire series represents a careful study of an important Mesozoic flora, and deals with external as well as internal characters, as far as the latter were available. The author is to be congratulated upon the successful completion of the botanical part of his work. The study of cuticles as a means of identification of fossil plants has received due consideration.—A. C. NOÉ.

American Ferns. By EDITH A. ROBERTS and JULIA R. LAWRENCE. New York: Macmillan & Co., 1935. Pp. viii+98. figs. 41. $2.50.

Descriptions of a number of species and suggestions for their wider use in landscape and general garden plantings are presented. A readily workable key of identification of a number of species as they appear throughout the year is of special interest. Suggestions are made for growing plants from spores and the times at which spores of various species may be obtained are given. The illustrations are numerous and of unusual clarity and excellence.—E. J. KRAUS.

Über die Beteiligung Kalkablagernder Pflanzen bei der Bildung Südbayerischer Tuffe. "Bibliotheca Botanica" No. 110. By J. WALLNER. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung, 1934. Pp. 30. pls. 2. RM 14.

Some of the coral reefs throughout geologic time, especially during the Mesozoic era, were formed by limestone deposits from plants. They are found in large quantities in the Alps, and a recent investigation of the formation of such vegetable coral reefs is reported in this book. The author made his observations in

southern Bavaria, in the foothills of the Alps. He investigated the form in which recent lime-secreting plants are acting and draws his conclusions from the activity of such plants during geologic time.—A. C. NOÉ.

Le Pansoma et la Géométrie de l'Enérgie. By A. C. LÉEMANN. Geneva: Georg and Company, 1935. Pp. 257.

Recent advances in physics and mathematics in the interpretation of matter, energy, and space will no doubt exert a profound influence upon philosophic thought. Those who are seeking some unifying principle in the diverse phenomena of nature, and those who are trying to interpret the complexities of our universe on some simple fundamental basis, will naturally turn to the newer units of matter and energy, and to the new space geometry as a point of departure. An attempt at such a simple explanation of a great range of phenomena has been made by DR. A. C. LÉEMANN of the University of Geneva, who derives the basis of his philosophy from the quantization of energy developed by PLANCK, and from the space geometry of EINSTEIN. The important step forward taken by LÉEMANN is essentially the geometrization of the quantum. No one has thought of the quantum as having three-dimensional space relations. But LÉEMANN argues from the identity of matter and energy, that if matter, one form of energy, is conceived to be three-dimensional, why should not the quantum itself be a three-dimensional energy unit? This three-dimensional unit he calls the *pansome.* It is indestructible, indivisible, conceived to be bounded sharply by pansomic surfaces, has an absolute and constant energy content, and has the power to manifest but one fundamental type of variability, that of changing its volume with its environment. Using this geometrized unit, LÉEMANN attempts to show how it is possible to give simple explanations of a great variety of natural phenomena. The fields of application will be briefly stated. He first takes up fundamental physical phenomena, energy, protons and electrons, temperature, ether, inertia, electricity, magnetism, radiations, etc. Then he passes to problems of cosmology, such as expansion of the universe, nebulae, creation of matter, conditions in the interior of stars, origin of stellar energy, etc. A little farther on he is considering affinity and valence, crystalline structure, properties of solution, adsorption, colloidal state, etc. From here it is but a step to vital phenomena, and he considers such familiar biological matters as karyokinesis, organogenesis, genetics, mutation, and evolution! Toward the close of the work, physiological processes come into the purview. Nerve conduction, muscular contraction, and the paratonic responses, both vectorial (taxies and tropisms) and non-vectorial (nasties, etc.) are considered. The last section enters the field of psychology and mental aberrations.

The author says that pansomism, in its essence, is only a kind of general psychology, which makes possible the interpretation of the marvellous harmony of nature in very simple but adequate terms. The reader may be disappointed at times because of the brevity of treatment; for although the work is extensive,

each section is rather short. On the other hand, this brevity of treatment of each topic makes it possible to stop frequently to meditate upon the applications of pansomism without too great wilderness of details. One should approach the work with open mind, and with a degree of skepticism in order most fully to enjoy the argument.—C. A. SHULL.

The Species of Tradescantia Indigenous to the United States. By EDGAR ANDERSON and ROBERT E. WOODSON. Jamaica Plain, Mass.: Arnold Arboretum, 1935. Pp. 132. pls. 12. $2.25.

During recent years the genus *Tradescantia* has been the object of much sustained investigation from the viewpoints of taxonomy, physiology, cytology, and genetics. And since the combination of cytology and genetics for the study of certain evolutionary processes has developed, *Tradescantia* has lent itself admirably as a subject for such work. This is not surprising, in view of the ease with which it can be grown from seeds or transplants, its ability to withstand varied environmental conditions, the long blooming period and ease of artificial pollination, and also because it has large chromosomes, and both diploid and tetraploid species and varieties occur in nature. Yet fundamental conclusions concerning the evolutionary importance of the cyto-genetic findings are not possible until they can be viewed against a background of intensive morphological and taxonomical research.

With these multiple needs in mind, the authors have made a serious effort to revise the native species of *Tradescantia* occurring north of Mexico. They have restricted the revision to the species indigenous to the United States for several reasons. This group of species, which are somewhat frequent in cultivation and available in the field, have been used, in the main, for most of the recent cytological and genetical investigations. From the taxonomic point of view this group, in which *T. virginiana* L. must be considered the type species, constitutes the typical element; and also morphologically the species indigenous to the United States with two exceptions fall into distinct groups of affinity which are limited in distribution to the region chosen. The species native to Mexico, the Antilles, and Central and South America are so little known that it was deemed wise to exclude them from this presentation.

Included are the taxonomic history, gross morphology, speciation, hybridization, study material, taxonomy, and an index. Under taxonomy there are a key to the species, descriptions, citations of synonyms and specimens, excluded species, and a list of exsiccatae. Twelve plates show various morphological and taxonomic characters of representative species.

This monograph will doubtless prove to be of considerable interest and value, not only to those who are engaged in research with the members of this genus, but to those also who are working on similar problems with other groups of organisms.—J. M. BEAL.

Problems in Soil Microbiology. By D. W. CUTLER and LETTICE M. CRUMP. London: Longmans, Green & Co., 1935. Pp. vii+104. $3.20.

The lectures on soil microbiology which were delivered by D. W. CRUMP on the Aberystwyth Foundation during the 1934–35 session have been modified and published as a monograph by CUTLER and CRUMP in the Rothamsted Agricultural Science Series. They record the results of work done in the department of general microbiology of the Rothamsted Experimental Station. The lectures present the soil as a medium to which the microbiological population has become adapted by selection through long ages, until now the soil is an "eminently suitable home" for the micro flora and fauna which inhabit it. The population is unspecialized to the extent that almost any decomposable substance will be attacked and used in the general economy of the population. The interrelations of the various types of organisms, the manner in which they influence one another's activity, form an important part of the work. The seven chapter titles indicate the scope of the monograph: The suitability of the soil for microorganisms, the bacterial population under field conditions, the relation of bacteria to nitrite, carbon dioxide production by soil, the growth of protozoa in pure culture, the behavior of protozoa in soil, and the interaction among the soil organisms. The work is a welcome addition to the popular agricultural monographs coming from the Rothamsted laboratories.—C. A. SHULL.

Praktikum der Zell- und Gewebephysiologie der Pflanze. By SIEGFRIED STRUGGER. Berlin: Gebrüder Borntraeger, 1935. Pp. xi+181. RM 8.50.

Laboratory Plant Physiology. By B. S. MEYER and D. B. ANDERSON. Ann Arbor, Michigan: Edwards Brothers, 1935. $1.75.

Two laboratory manuals of plant physiology have been published during recent months. One is by STRUGGER and the other by MEYER and ANDERSON. STRUGGER'S work is devoted to general cellular physiology, and presents 94 experiments, arranged in eleven topical sections.

The manual by MEYER and ANDERSON is a general work for classes in plant physiology. It is interleaved to permit taking of notes on observations. There are 132 experiments, arranged in eighteen sections with logical sequence. Both manuals should prove valuable in different types of courses.—C. A. SHULL.

Chronica Botanica. Vol. I. Edited by FR. VERDOORN. Leiden, Holland, 1935. Pp. 447.

A useful yearly publication, Chronica Botanica, made its first appearance this spring. The bulk of the volume is devoted to a review of all botanical departments in universities, gardens, museums, national societies, government bureaus, colonial departments of botany and agriculture; in short, to all local activities of a botanical character. The reports are in any of the five languages: English, German, French, Spanish, and Italian.

It is to be regretted that the questionnaire for the Chronica Botanica was not answered by a number of botany departments in large universities, especially in the United States; therefore the space given to individual institutions does not always correspond to the true importance of the same.—A. C. NOÉ.

Annual Review of Biochemistry. Stanford University Press, 1935. Pp. viii+639. $5.00.

Several years ago the Stanford University Press began the issue of an annual volume devoted to the recent advances in biochemistry. The fourth volume has just been issued. It contains 27 reviews, many in the fields of animal metabolism, a few in the fields of general or plant physiology. Among the latter may be mentioned the sections on permeability, biological oxidations and reductions, chemistry of the carbohydrates and glycosides, vitamins, plant pigments, the alkaloids, the mineral nutrition of plants, growth substances in plants, and the chemistry of bacteria. It is not possible for the reviewers to take into consideration all the literature produced in the past year or two in any of these fields; they must select the important facts from among the less important contributions. The writer feels that the responsible reviewers have been unusually successful in producing well written reviews that faithfully reflect the progress of biochemical science year by year. The series constitutes a remarkable record of the history of biochemistry during the period covered, and its importance will be increasingly evident as the series lengthens.

The editors of the series, J. MURRAY LUCK, C. L. ALSBERG, D. R. HOAGLAND, and C. L. A. SCHMIDT, deserve great credit for the success of this venture into the summarizing field. The Annual Review has become indispensable to physiologists who want to keep abreast of current thought and oriented to the direction of the main advances. It is gratifying that the series has met with the approval of workers, and that its successful continuation is assured.—C. A. SHULL.

Flora of Iceland and the Faeroes. By C. H. OSTENFELD and JOHS. GRÖNTVED. Copenhagen: Levin and Munksgaard, 1934. Pp. xxiv+195. maps 2.

The late Professor C. H. OSTENFELD had for several years, prior to his death in 1931, made preparations for a Flora of Iceland and the Faeroes, completing the text as far as the Leguminosae. The remainder of the text, including keys, glossary, and index, has since then been completed by JOHS. GRÖNTVED, and the whole issued as a handy volume of little more than pocket size. The text is in English. The style is simple and direct, making a special appeal "as a field-book for amateurs and botanists" who might wish to become acquainted with the flora of these islands. A large number of genera and even many species common in continental North America and known to most of our American students are represented. A special attempt at completeness in listing vernacular names is evident, the scientific names being regularly accompanied with the Icelandic or Faeroesian equivalents. Altogether, the book seems likely to prove exceptionally

usable, unless perhaps in the case of certain genera (for example, *Taraxacum*, *Hieracium*) where a number of apparently dubious specific segregations have been admitted.—E. E. SHERFF.

Les zoocécidies des plantes de l'Amérique du Sud et de l'Amérique Centrale. By C. HOUARD. Paris: Hermann & Co., 1933. Pp. 519. figs. 1027. map 1.

An important recent work on galls is the volume by HOUARD, of the University of Strasburg. It deals with the insect and other animal galls known so far for Central and South American plants. The volume is largely in the nature of a compendium for reference.

Only three pages are needed for the galls peculiar to the vascular cryptogams, as compared with about 433 pages for the phanerogams. The text is arranged in botanical sequence, a great convenience to users, who are primarily botanists. Each kind of gall or gall effect is described in detail and in numerous cases illustrated with a simple but instructive figure. General facts of classificatory significance are largely systematized and represented with suitable abbreviations. Thus *acrocécidie* (gall terminal) is indicated by Acrc. as distinguished from Plrc. for *pleurocécidie* (gall lateral). Whether the gall deforms the fruit, capitulum, inflorescence, flower, tip of stem, bud, or other part of the plant is shown by Ac.fr., Ac.cp., Ac.inf., Ac.fl., Ac.ti., etc. Abbreviations such as M.C. and M.T. indicate whether the metamorphosis of the animal organism concerned occurs in the gall or in the earth.

An extensive bibliographic index accompanies the work and two other indexes, one to the plants and one to the causal animal organisms, are given.—E. E. SHERFF.